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Isolation of five new monosomic alien addition lines of *Gossypium australe* F. Muell in *G. hirsutum* L. by SSR and GISH analyses

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Abstract

Gossypium australe F. Muell (2n = 2x = 26) is a wild perennial species possessing agronomic useful traits that would be interesting to introgress into *G. hirsutum* L. (2n = 4x = 52), the main cultivated cotton species. To isolate monosomic alien addition lines (MAALs) of *G. australe* in *G. hirsutum*, the [2(*G. hirsutum* × *G. australe*) × *G. hirsutum*] pentaploid (2n = 5x = 65) was backcrossed as male parent to *G. hirsutum*. Analysis of 42 BC_1 plants and seven alien addition lines, already available, with 150 SSR markers developed from *G. hirsutum* revealed a cross-species amplification rate of 100% and a polymorphism rate of 56%. Eighty polymorphic SSR markers generated 87 *G. australe*-specific loci that have been assigned by a hierarchical cluster analysis to 13 linkage groups corresponding to the 13 chromosomes of *G. australe*. Analysis by SSR markers and genomic *in situ* hybridization of the self-progeny of disomic alien addition lines, backcross progeny of the pentaploid, allowed the isolation of five new MAALs.

Key words: cotton — aneuploid — chromosome

Australian wild *Gossypium* diploid species (2n = 2x = 26) harbours numerous economically useful characteristics such as delay of gossypol gland morphogenesis in the seed and resistance to biotic stresses that would be interesting to introgress into the main tetraploid (2n = 4x = 52) cultivated species (Brubaker et al. 1996, McFadden et al. 2004). One strategy is to produce monosomic alien addition lines (MAALs) by repeated backcrossing of an allohexaploid (2n = 6x = 78) obtained from colchicine-doubled triploid (2n = 3x = 39) hybrid issued from a cross between a diploid species and the tetraploid cultivated species (Ahoton et al. 2003, Becerra Lopez-Lavalle and Brubaker 2007). MAALs are useful genetic materials for alien gene transfer as well as for the study of genomes' structure and pathways of evolution (Brubaker et al. 1999, Mergeai 2006).

They are valuable materials for gene identification (Becerra Lopez-Lavalle et al. 2007), gene tagging (Bai et al. 1994), chromosome mapping (Suen et al. 1997, Brubaker and Brown 2003), microdissection and microcloning for establishment of specific-chromosome DNA libraries (Potz et al. 1996) and comparative mapping (Chen et al. 1997).

Numerous studies focusing on the production and exploitation of alien addition lines of diploid *Gossypium* species in *G. hirsutum* have been achieved (Hau 1981, Rooney et al. 1991, Mergeai et al. 1993, Ahoton et al. 2003; Brubaker and Brown 2003; Becerra Lopez-Lavalle and Brubaker 2007). Isolation of MAALs consisting of identification of each alien chromosome and identification of the plants carrying only one alien chromosome was usually based on classical cytogenetic analysis combined with morphological survey. These techniques are however time-consuming and unreliable. Thus, despite many efforts, no isolation of a complete set of diploid *Gossypium* MAALs in *G. hirsutum* has been reported so far. However, molecular genetic markers and molecular cytogenetic techniques have opened a new avenue to identify and use MAALs to transfer agronomical traits of interest (Fedak 1999, Kato et al. 2005).

Because of their ease of generating numerous loci, AFLP markers have been used (Brubaker and Brown 2003, Becerra Lopez-Lavalle and Brubaker 2007) to screen the first and second generation aneuploids of 2(*G. hirsutum* × *G. australe*) and 2(*G. hirsutum* × *G. sturtianum*.H.Willis) hexaploids backcross progeny. This research identified 13 chromosomes of both diploid species but did not address the issue of their isolation in the same genetic background. Considering that numerous SSR markers have been developed and mapped onto *G. hirsutum* chromosomes (Lacape et al. 2003), Ahoton et al. (2003) took advantage of their cross-species transportability to unravel the homoeological relationships between *G. australe* and *G. hirsutum* chromosomes and isolate 6 of the possible 13 MAALs carrying the *G. autrale* chromosomes homoeologous to *G. hirsutum* chromosome pairs c3-c17, c6-c25, c7-c16, c9-c23, c10-c20 and c12-c26.

To isolate the remaining 7 *G. australe* chromosomes in the background of *G. hirsutum*, this study aims to: (i) define a linkage group for each *G. australe* chromosome by analysing the first backcross progeny of the pentaploid [2(*G. hirsutum* × *G. australe*) × *G. hirsuutm*] with SSR markers developed in *G. hirsutum* or derived from *G. arboreum*; (ii) confirm by genomic *in situ* hybridization (GISH) analysis the equivalence of these linkage groups to *G. australe* chromosomes.

Materials and methods

Plant material: The Fig. 1 describes the scheme to develop MAALs of *G. australe* in *G. hirsutum*. The pentaploid (2n = 5x = 65) was backcrossed as male parent to *G. hirsutum* cultivar 'Stam F' to produce BC_1 seeds, which were sown during the summer in the glasshouses of Gembloux Agricultural University. All the BC_1 seeds were derived from one pentaploid plant that has been multiplied by grafting onto *G. hirsutum* as root-stock. The grafting generates enough plants to carry out the hybridization programme while allowing to avoid the considerably difficulty task of producing pentaploid seeds and making them germinate and grow in viable plants. We selected 42 BC_1 plants that had the morphological characters (growth habit, colour and shape of the leaves, colour of the flowers, relative position of the stigma and the staminal column, size and shape of the bolls) and phenological traits (days to flowering and days to harvest) far removed from that of *G. hirsutum* and from the seven alien addition lines (six MAALs and one DAAL) already isolated by Ahoton et al. (2003). It is assumed that this morphological difference would allow the recovery of plants with alien additional chromosomes not yet isolated.

DNA extraction, PCR amplification and gel electrophoresis: Total genomic DNA was extracted from fresh leaves in a 300 µl lysis buffer (100 mM Tris-HCl pH 8.0, 1.5 M NaCl, 20 mM EDTA pH 8, 2% MATAB, 0.5% sodium sulphite, 1% polyethylene glycol 6000), incubated 60 min at 72°C, extracted with chloroform isoamyl alcohol (24 : 1) and precipitated with isopropanol. PCR amplifications were performed following Nguyen et al. (2004) in 10 µl reaction mixture containing 25 ng of DNA, 0.2 mM dNTP, 2 mM MgCl2, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.2 µM of radio-labelled primer, 0.1 µM of unlabelled primer and 0.6 U *Taq* DNA polymerase. All PCR amplifications were performed using a PTC100 Thermal Cycler (MJ Research, Waltham, MA, USA). Radio-labelled PCR products were obtained by 5′-end labelling one of the primers with [γ^{33}P] dATP using T4 polynucleotide kinase (Fermentas GmbH, Germany). After adding 20 µl of loading buffer (98% formamide, 10 mM EDTA, bromophenol blue, xylene cyanol) to the PCR reactions, the mixture was denaturated at 94°C for 3 min, and 5 µl of each sample was loaded onto a 5% (w/v) polyacrylamide gel containing 7.5 M urea and electrophoresed in 1× TBE buffer at 55 W for 1 h 20 min to 1 h 50 min. After the run, gels were transferred onto Whatman 3MM paper, dried at 80°C for 20 min in a gel dryer and exposed to X-ray films (Kodak, X-Omat, Sigma-Aldrich, Rochester, NY, USA) for 2–3 days.

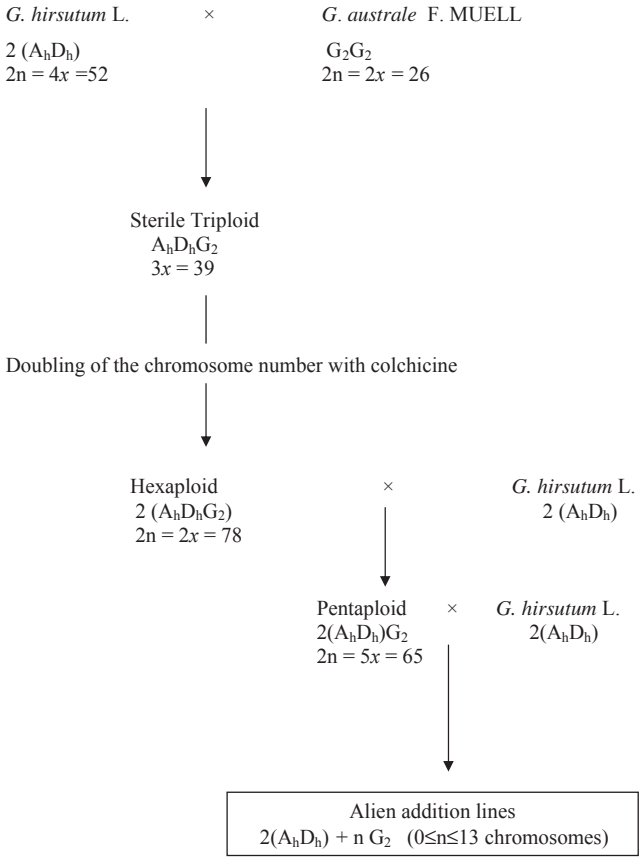

Fig. 1: Development scheme of alien addition lines of *G. australe* in *Gossypium hirsutum*

SSR genotyping: From the website http://www.cottonmarker.org, 150 SSR markers mapped to 26 chromosomes of *G. hirsutum* and covering the whole genome have been selected according to their position on three maps (Nguyen et al. 2004, Frelichowski et al. 2006; Han et al. 2006), also available from this website. The establishment of the 13 homoeologous chromosome pairs of *G. hirsutum* was based on Wang et al. (2006). The 150 markers were first tested on *G. hirsutum* and *G. australe* to detect polymorphism. Those showing unambiguous polymorphism were retained for further screening of the BC_1 generation. An SSR locus was considered *G. australe*-specific when it yielded a PCR fragment simultaneously present in *G. australe* and in the bispecific hexaploid but absent from *G. hirsutum*. Two RFLP-derived SSR markers [previously known as producing polymorphic bands between *G. australe* and *G. hirsutum* (Lacape, unpublished data)] UGA13 and UGA51 drawn from the probes GateCD12 and pGH322, respectively, (Rong et al. 2004) were used. Except for these two markers, positions and sequences of all other SSRs are available at the website given above.

Genomic *in situ* hybridization: To confirm the equivalence of linkage groups to *G. australe* chromosomes and to determine whether these chromosomes were recombined with *G. hirsutum* chromosomes, GISH analysis was realized using a protocol adapted from D'hont et al. (1995). Mitotic chromosomes were prepared from root tip cells. Total genomic DNA of *G. hirsutum* was labelled with digoxigenin-11-dUTP (Roche Applied Science, Mannheim, Germany) and total genomic DNA of *G. australe* with biotin-16-dUTP (Roche Applied Science) by the nick translation method following the manufacturer's instructions. Chromosomes were counterstained with 4′,6-diamidino-2-phenylindole (DAPI), and slides were analysed using a Leica epifluorescence microscope with filter blocks for DAPI, fluorescein isothiocyanate-5 (FITC) and Texas red. Photographs were taken with a JVC KY-F58E (JVC, Yokohama, Japan) camera. Images were processed using the Microsoft Photo Editor software.

Statistical tests: To assemble the SSR loci markers in linkage groups from the BC_1 genotyping results, a hierarchical cluster analysis including all individuals carrying at least one marker was performed with the program SAS System (SAS Institute Inc., Cary, NC, USA). A matrix of dissimilarities between markers has been computed. For any two markers, the dissimilarity has been defined as 1-*j*, *j* representing Jaccard coefficient (Everitt and Rabe-Hesketh 1997). This coefficient is the ratio of the number of plants with loci generated by two given markers to the number of plants with at least one locus generated by one of the two markers. The markers have then been clustered using the hierarchic average linkage algorithm (Manly 1994).

Results

Transferability and polymorphism of the SSR markers

All of the 150 SSRs amplified *G. australe* DNA but only 84 revealed clear polymorphism with *G. hirsutum*. These 84 markers produced overall 89 polymorphic loci. Among the 84 markers, 81 produced a single polymorphic locus. Two

markers (BNL1227 and BNL3992) yielded two polymorphic loci, and one marker (BNL3537) generated four loci.

Establishment of the *G. australe* linkage groups

Among the 89 *G. australe*-specific loci, two loci generated by the markers CIR047 and CIR110 did not appear in the hexaploid nor in any of the 42 BC_1 plants. Eighty-six polymorphic loci were unequivocally clustered in 13 linkage groups (Table S1–S2) assumed to correspond to the 13 chromosomes of *G. australe*. The remaining marker CIR280 was the only one that could not be reliably assigned to a linkage group. Table 1 shows the assignment of all the polymorphic loci, except for CIR280, to the thirteen linkage groups.

Isolation of new MAALs and assignment of the marker CIR280

Assuming that the 13 linkage groups did correspond to the 13 complete *G. australe* chromosomes, segregation of the *G. australe* chromosomes in the pentaploid progeny and in the alien addition lines already isolated is given in Table 2.

None of the seven *G. australe* chromosomes remaining to be isolated (c1, c2, c4, c5 c8, c11 and c13) was in a monosomic addition configuration. The five plants with only one complete alien chromosome (#1, #6, #9, #24 and #29) were among the already isolated MAALs.

With the unsuccessful recovery of new MAALs from the pentaploid backcross progeny (BC_1), we had to proceed from the backcross progeny (BC_2) or from the self-progeny (BC_1S_1) of selected multiple alien addition plants (BC_1) carrying chromosomes that were not yet isolated. Even though the backcross progeny would be more recommended than the self-progeny, we decided to isolate the remaining MAALs from the self-progeny given that we already had self-progeny seeds of each of the BC_1 plants.

To isolate the chromosomes c2, c4, c5 and c8, the disomic alien addition plants #13 (carried c2 and c9), #20 (c4 and c6), #7 (c5 and c6) and #10 (c8 and c9) were selected. As the only plant carrying c13 (#26) showed complete self-sterility, we chose to isolate this chromosome from the DAAL G_2XII already isolated by Ahoton et al. (2003). The *G. australe* c11 could have been isolated from the plant #5 (carried c10, c11 and c12) or #34 (carried c10 and c11) and *G. australe* c1 from #17, but all these three plants were completely self-sterile. The self-progeny of plants #13, #20, #7, #10 and G_2XII was analysed with SSR markers (Tables S3–S7). The results are presented in Table 3. They confirmed the validity of the established linkage groups as markers already assembled in a linkage group always cosegregated together. In some cases, however, transmitted alien chromosomes were broken (absence of some SSR markers).

Analysis of the self-progeny of plant #7 showed that CIR280 always cosegregated with *G. australe* c5 (Table S6). This cosegregation allows finally to assign CIR280 to this chromosome.

Genomic *in situ* hybridization analysis performed on self-progeny of the disomic addition plants #13, #20, #7, #10 and G_2XII carrying only one linkage group confirmed the correspondence of the five linkage groups with *G. australe* chromosomes (Fig. 2). The GISH analysis of the derivatives of plant #20 carrying only the marker CIR280 showed no *G. australe* segment, recombined or not.

Segregation pattern of CIR 280 in the BC_1 generation and in #20 self-progeny

The high frequency of CIR280 in the BC_1 generation (17 plants out of 42) suggests that this marker represents a chromosome fragment preferentially transmitted or recombined in the *G. hirsutum* background genome. The chi-square test ($\chi^2 = 1.52$; $P = 0.217$) is in agreement with an expected transmission ratio of 1 : 2 indicative of a recombination that would have taken place in the hexaploid.

The hypothesis that segregation of CIR280 in the pentaploid progeny is an indication of the introgression of a *G. australe* fragment is reinforced when its distribution in plant #20 self-progeny is considered (Table 3). Thirty-eight plants out of 46 (82%) carry the marker CIR280. This number is equivalent to the expected 3 : 4 ratio in the transmission frequency ($\chi^2 = 1.42$; $P = 0.75$) if plant #20 was hemizygous.

Sterility in the BC_1 generation and assignment of morphological characteristics to *G. australe* chromosomes

Five years after sowing, five BC_1 plants still showed complete self-sterility. Three of these plants (#16, #26 and #17) were those carrying the maximum chromosome numbers (respectively 5, 6 and 7 chromosomes). However, sterility was not only associated with the number of supernumerary chromosomes. The only disomic (#34) and trisomic addition plants (#5) that showed complete self-sterility had the particularity to carry *G. australe* c11. Thus, this chromosome seems to carry genes causing sterility. All the fertile plants carrying *G. australe* c6 produced brown lint, and all the plants carrying the complete c7 showed pink flower. This suggests that c6 and c7 respectively carry gene(s) for brown lint and pink flower.

Discussion

Transferability of SSR markers

Transferability of SSR markers is based on the conservation in related species of the regions flanking the microsatellite sequences. The 100% rate of cross-species amplification of *G. hirsutum* and *G. arboreum*-derived microsatellite in *G. australe* showed a great conservation of the priming sequences between the A- and D-genome and the G-genome of the australian diploid species despite their evolutionary divergence that is reckoned to date back 6–11 million years ago. Altaf-Khan et al. (2006), analysing 255 primers derived from *G. hirsutum* on two *Gossypium* G-genome species (*G. australe* and *G. nelsonii*) and on the two putative diploid progenitors of the allotetraploid, found out that 19% of the primers did not amplify the diploid species. But they did find that 17% of these markers did not amplify their *G. hirsutum* DNA either. Thus, the non-amplification of these SSR is less likely because of the non-conservation of the priming regions than to the conditions of the PCR which were a touchdown method with an annealing temperature of 65°C during the first cycles.

The good transferability of SSR is also supported by results obtained in other genera: Guyomarc'h et al. (2002) reported that 92% SSR markers developed from *Aegilops tauschii* amplified the bread wheat *Triticum aestivum*.

Among the 150 markers sorted out, 84 (56%) showed at least one *G. australe*-specific DNA fragment. Altaf-Khan et al.

Table 1: Assignment of the polymorphic SSR loci to *Gossypium australe* chromosomes

G. australe chromosome	c1	c2	c3	c4	c5	c6	c7	c8	c9	c10	c11	c12	c13
Gossypium hirsutum homoelogous pair chromosome	c1–c15	c2–c14	c3–c17	c4–c22	c5–c19	c6–c25	c7–c16	c8–c24	c9–c23	c10–c20	c11–c21	c12–c26	c13–c18
	NAU2083	CIR202 (195)	MUSS073 (210)	**BNL3992a (315)**	NAU2014 (219)	BNL3359 (187)	BNL1694 (230)	BNL3257 (172)	BNL2847 (220)	CIR171 (218)	UGA51 (157)	BNL1227a (170)	BNL2571 (240)
	CIR411 (188)	BNL3989 (245)	MUCS620 (237)	CIR253 (190)	**BNL3992b (135)**	BNL1047 (155)	BNL1604 (104)	CIR119 (274)	BNL1317 (195)	NAU1182 (228)	CIR196 (210)	BNL1227b (172)	UGA13 (245)
	MUSS422	JESPR231	BNL3413 (112)	BNL3995 (170)	NAU2231 (216)	**BNL3436 (190)**	NAU2186 (144)	BNL3556 (134)	CIR372 (168)	BNL3563 (210)	NAU2110 (315)	BNL1679 (137)	MUSS140 (206)
		BNL1897 (118)	BNL2443 (124)	NAU2121 (235)	BNL2448 (127)	BNL1440 (210)	NAU2432 (151)	CIR289 (221)	BNL4053 (188)	BNL119 (130)	MUSS092 (303)	**BNL3537c (170)**	
		CIR228 (192)	CIR180	NAU2474 (178)		CIR407 (157)		NAU2439 (138)	BNL2590 (193)	CIR187 (290)	NAU1366 (219)	**BNL3537d (177)**	
		CIR210 (102)		BNL4030 (106)		BNL1061 (155)		BNL252 (165)	BNL597 (188)		BNL1408 (176)	BNL1673 (185)	
		CIR292 (202)		CIR122 (138)		BNL3103 (180)		MUSS500 (226)	MUSS22 (128)			BNL3599 (175)	
		BNL3259 (198)		CIR218 (185)		BNL2884 (175)		BNL3084 (166)	BNL686 (140)			BNL3261 (190)	
				NAU2291 (214)		**BNL3537a (139)**						CIR272 (223)	
				NAU869 (180)		**BNL3537b (141)**						**BNL3436 (190)**	
				MUSB1093 (255)		BNL2569 (152)							
				NAU2235 (146)									
Total number of polymorphic loci	3	8	5	12	4	11	4	8	8	5	6	10	3

Chromosome designation and homoeology relationships of *G. australe* chromosomes with tetraploid cotton chromosome pairs are based on the positions of loci on the published *G. hirsutum* maps (cf.http://www.cottonmarmer.org).

Markers in bold are duplicated in different *G. australe* chromosomes. The letters a, b, c and d distinguish loci of a same marker.

Table 2: Distribution of the *Gossypium australe* linkage groups in the BC_1 aneuploid plants and in previously isolated MAALs

Linkage groups	Pentaploid self-progeny[1]																													Alien addition lines already isolated by Ahoton et al. (2003)						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	G_2I	G_2II	G_2III	G_2IV	G_2V	G_2VI	G_2XII
1																	x																									
2								x									*	x																								
3								x										*								x															
4																x				x	*												x								
5							x										x					x				x															x
6							x	x				x			x					x		x			*	x								x							
7														*			*						x				*				x					x					
8			*							x		x			x	x														x	x										
9									x	*			x	*	*	*	x		*									*	*			*	x		x						
10		x		x		x	x									x	*	x												*				x							
11					x																					x					*			x							
12				*	*									*		x	*								x	x	*	*	x			x	x				x				
13																										x															
CIR280		+				+	+		+			+				+				+		+		+		+	+		+	+	+	+	+		+					x

[1]Only BC_1 plants that carried at least one *G. australe*-allele are mentioned.
X, Complete *G. australe* chromosome; *, Partial *G. australe* chromosome (at least 1 locus is absent); +, Presence of one *G. australe*-specific locus.

(2006) found out that 68% (85/255) of the SSRs that amplified the diploid species were polymorphic.

Statistical evidences that a recombination occurred in the hexaploid

CIR280 cosegregating with c5 in plant #7 self-progeny has been reliably assigned to this chromosome. Its irregular segregation in the BC_1 derivatives (that had first prevented its assignment to any linkage group) and in plant #20 self-progeny is statistically in accordance with the hypothesis of an allosyndetic recombination that would have occurred in the hexaploid. It is known that in the course of MAALs production, intergenomic exchanges may occur at the hexaploid or pentaploid stages at a rate depending on the pairing affinities between the two genomes of the bispecific hybrid (Beasley 1942, Brown and Menzel 1952, Mergeai 2006). In the present case, this heterogenetic recombination cannot have occurred in the pentaploid, because if this was the case, because of the low frequency of allosyndetic recombinations, it would have been unlikely to observe such a high transmission frequency of the marker in the BC_1 generation.

In the analysis of 11 BC_1 multiple addition lines of *G. sturtianum* in *G. hirsutum,* Becerra Lopez-Lavalle and Brubaker (2007) obtained a linkage group designated Sturt-B that could not be linked to any of the 13 AFLP-linkage groups assumed to correspond to the 13 chromosomes of *G. sturtianum* and hypothesized that this linkage group represented an introgressed segment. Beasley (1942) indicated that with the hexaploid involving *G. sturtianum* 39 pairs of chromosomes are regularly observed, although some are usually involved in tetravalents. Brown and Menzel (1952), Da silva et al. (1975), however, found these associations are so rare, and they suggested to consider those associations as virtually negligible and predicted this stage to be of no interest for introgression. On the contrary, Louant et al. (1977) argued that the hexaploid, being the only balanced, euploid, and fertile stage in the development scheme of MAALs should be considered as an important stage for intergenomic exchanges instead of being considered useless for introgression and only necessary for restoring the fertility. He predicted that selfing the hexaploid for several generations would increase recombination potential.

Although there is no general agreement on the importance of allosyndetic recombinations in the hexaploid stage and its possible enhancement with the increase in selfing-generations, our results confirm that introgression can take place in the *G. hirsutum* × *G. australe* hexaploid.

The fact that the fragment carrying the marker CIR280 was not detected by GISH indicates that it is likely of a small size and highlights one of the limits of this technique which is the difficulty of detecting introgression of small chromosome fragments (Pedersen and Linde-Laursen 1995). Resolution of GISH depends on cytological targets (interphase nuclei, metaphase chromosomes, meiotic pachytene chromosomes, extended DNA fibres etc.) among which mitotic metaphase chromosomes, the most condensed chromosomes, have the lowest resolving power (Jiang and Gill 2006). To our knowledge, this resolving power has not yet been studied in the genus *Gossypium* but in the genus *Hordeum*, for example, this distance has been estimated to a minimum of 5–10 Mb (Pedersen and Linde-Laursen 1995).

Table 3: Segregation of SSR markers in the disomic addition lines self-progeny

	Gossypium australe chromosomes carried by the disomic alien addition line plant	Number of plants with the *G. australe* chromosome[1]	Number of plants with the marker CIR280	Total number of plants analysed
#20 self-progeny[2]	c4	14	38	46
	c6	11		
#13 self-Progeny	c9	12		32
	c2	16		
#10 self-progeny	c9*	15		30
	c8	12		
#7 self-progeny[2]	c5	8	5	26
	c6	5		
G_2XII self-progeny	c13	3		11
	c5	0		

[1]Some of the plants carried incomplete *G. australe* chromosomes (not carrying all the markers characteristic of the *G. australe* chromosome).
[2]Plants carrying the marker CIR280.

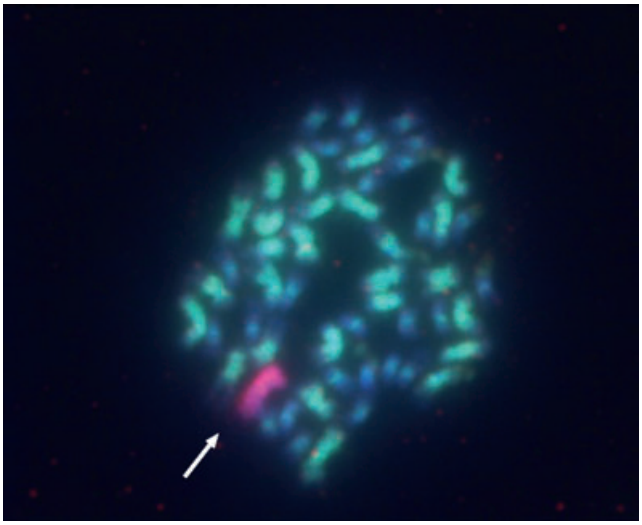

Fig. 2: Identification of *Gossypium australe* chromosomes in *G. hirsutum* background. Metaphase of a self-progeny plant of 20 with the *G. australe* chromosome c4. The arrow indicates the *G. australe* additional chromosomes. Large green and small blue chromosomes represent respectively the A- and the D-genome of the *G. hirsutum* background

Assignment of genes of sterility and of morphological characteristics to *G. australe* chromosomes

The only disomic (#41) and trisomic addition plants (#5) that showed complete self-sterility had the particularity to carry *G. australe* c11. The fact that sterility of an alien addition plant can be chromosome specific was demonstrated in alien addition lines of barley in wheat. The alien addition line carrying the barley chromosome 5 (1H) could not be generated because of the presence of one or more genes causing sterility when present in wheat (Islam et al. 1981).

Becerra Lopez-Lavalle and Brubaker (2007) associated the traits 'mauve flowers' and 'brown lint colour' to the *G. australe* chromosomes carried by their designated MAALS Aust-F and Aust-A, respectively. So, it can be stated that these two chromosomes are respectively equivalent to our *G. australe* c7 and c6.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Segregation of SSR polymorphic loci in the pentaploid progeny and in alien addition lines already isolated

Table S2. The cluster procedure of the Average Linkage Cluster Analysis

Table S3. Segregation of SSR markers in #24 self-progeny

Table S4. Segregation of SSR markers in #14 self-progeny

Table S5. Segregation of SSR markers in #11self-progeny

Table S6. Segregation of SSR markers in #7 self-progeny

Table S7. Segregation of SSR markers in G_2XII self-progeny

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