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Cytogenetics of the 'glandless-seed and glanded-plant' trait from *Gossypium sturtianum* Willis introgressed into upland cotton (*Gossypium hirsutum* L.)

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With 3 figures and 2 tables

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Abstract

In order to introgress the 'glandless-seed and glanded-plant' trait from *Gossypium sturtianum* Willis ($2n = 2x = 26$, C_1 genome) into the cultivated upland cotton *Gossypium hirsutum* L. ($2n = 4x = 52$ $(AD)_1$ genome), two trispecific hybrids have been created using either *Gossypium thurberi* Torado ($2n = 2x = 26$, D_1 genome) or *Gossypium raimondii* Ulbrich ($2n = 2x = 26$, D_5 genome) as bridge species. The cross of both trispecific hybrids by *G. hirsutum* produced the first backcross progenies (BC1). Cytogenetic analysis showed that the trispecific hybrids had 52 chromosomes, their chromosome configurations at metaphase I (MI) being 15.07I + 15.34II + 0.93III + 0.69IV + 0.26VI in *G. thurberi* × *G. sturtianum* × *G. hirsutum* (TSH) and 14.42I + 17.03II + 0.82III + 0.15IV + 0.07VI in *G. hirsutum* × *G. raimondii* × *G. sturtianum* (HRS), respectively. Among six BC1 plants analysed, the only plant expressing the 'glandless-seed and glanded-plant' trait had 52 chromosomes and a meiotic configuration of 5.26I + 20.61II + 0.69III + 0.77IV at MI. Pollen fertility was 2.90% in TSH, 8.97% in HRS, and ranged from 0% to 40.28% in the BC1 progenies. The introgressed BC1 plant is perennial in growth habit. It can be used in breeding programmes aiming at the introgression of the 'glandless-seed and glanded-plant' trait into a cultivar of upland cotton.

Key words: *Gossypium* spp. — cytogenetic analysis — gossypol glands — interspecific hybridization

The genus *Gossypium* contains about 50 diploid and tetraploid species indigenous to most of the tropical regions of the world (Fryxell 1965, Fryxell et al. 1992). Similar genomes are designated by the use of the same capital letter and closely related genomes are distinguished by a numerical subscript after each letter of that class (Beasley 1942). The diploid species ($2n = 2x = 26$) are divided into eight different cytotypes designated by A, B, C, D, E, F, G and K, based on the chromosome pairing relationships (Beasley 1940, Beasley 1942, Gorham and Young 1996). The chromosomes of A and B genomes are intermediate in size between the small chromosomes of D genomes and the large chromosomes of C, E, F, G and K genomes (Endrizzi et al. 1985, Gorham and Young 1996). Cultivated types belong to four species, of which *Gossypium herbaceum* and *Gossypium arboreum* are diploid, while *Gossypium hirsutum* and *Gossypium barbadense* are tetraploid. The tetraploid species ($2n = 4x = 52$) contain two distinct subgenomes which are related to the A genome of the Asiatic cultivated diploid species and the D genome of the American wild diploid species (Geever et al. 1989). The main cultivated species, *G. hirsutum* ($(AD)_1$ genome, also designated A_hD_h), provides 95% of the world production of lint. One of the main traits of *Gossypium* is the presence of pigment glands throughout the plant (Altman et al. 1990). Gossypol, a triterpenoid aldehyde, and its derivatives are the predominant pigments in cotton glands. These compounds have insecticidal, antimicrobial, antifertility and toxic properties (Stipanovic et al. 1984, Fisher et al. 1988, Percy et al. 1996).

Cotton is not only the most important fibre crop in the world, it is also the second best potential source of plant proteins after soybean, and the fifth best oil-producing plant after soybean, palm-tree, colza and sunflower (Texier 1993). Cottonseed kernels contain about 38% lipid and 39% proteins. The incorporation of cotton flour into animal feed gave very good results, and studies carried out in Africa, India, Peru, France and the USA showed that cottonseed proteins can also be used to improve nutritional and functional properties of human food (Marquié 1987, Bourély 1987, Alford et al. 1996). The utilization of this important protein and oil source is still limited because gossypol is noxious for monogastric animals, and particularly toxic to humans. Various procedures are used by the cottonseed-crushing industry to eliminate gossypol from the seed derivatives (oil, cakes and by-products), but they alter proteins, decrease the nutritional value of cotton flour, and increase the extraction costs of oil, cakes and by-products (Rahma and Rao 1984; Wan et al. 1995). Completely glandless cotton was first identified by McMichael (1954) but the glandless varieties bred subsequently were highly susceptible to phytophagous insects. Ideal cultivated cotton plants should have 'glandless-seed' for complete use in food and feed, and 'glanded-aboveground parts' to resist insects pests.

The gossypol content of cotton (*Gossypium* spp.) is controlled by at least six independent loci, namely gl_1, gl_2, gl_3, gl_4, gl_5 and gl_6 (Pauly 1979). The formation of gossypol glands in the cultivated upland cotton is controlled by two main alleles Gl_2 and Gl_3 (McMichael 1960), located on the homoeologous chromosomes 12 (A genome) and 26 (D genome), respectively (Endrizzi et al. 1985, Samora et al. 1994). Seed gossypol content is determined mainly by the Gl_2 allele (Lee 1965, Pauly 1979, McCarty et al. 1996). Among the 50 species of *Gossypium*, the 'glandless-seed and glanded-plant' trait is found only in some Australian wild species. In these plants, the genes involved in gossypol gland formation appear to be controlled by a repressive mechanism which acts until the cotyledons open and the young plantlets begin to form chlorophyll (Mergeai 1992). To introgress this useful trait from the Australian wild diploid species *Gossypium sturtianum* Will. (C_1 genome) into the main cultivated tetraploid cotton *G. hirsutum*, we created two differ-

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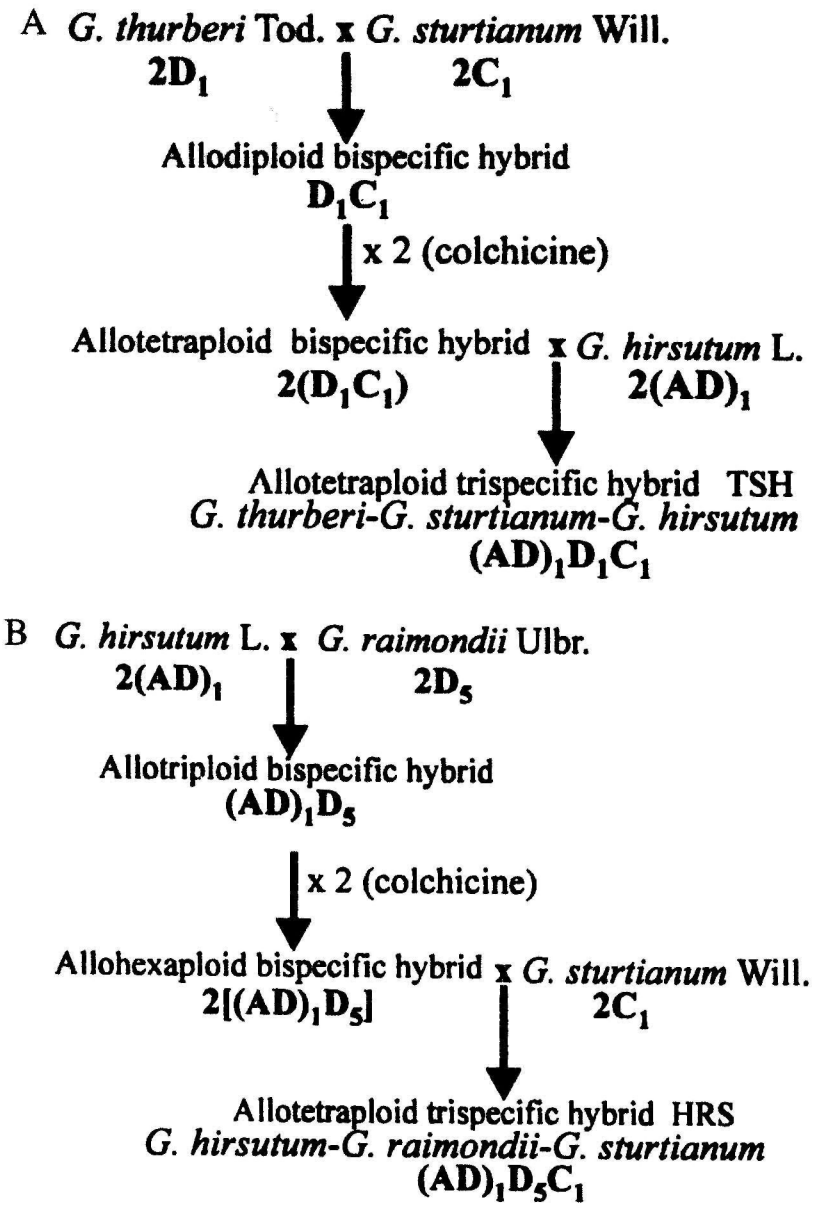

Fig. 1: Development of the trispecific hybrids. A. Scheme to create TSH (*G. thurberi* × *G. sturtianum* × *G. hirsutum*). B. Scheme to create HRS (*G. hirsutum* × *G. raimondii* × *G. sturtianum*). Genomic formulae are given in bold

ent trispecific hybrids to confront all the chromosomes of *G. sturtianum* with the chromosomes of *G. hirsutum* (Mergeai et al. 1995). An evaluation of chromosome associations in such complex hybrids is of importance to the breeder, since it indicates the feasibility of using genetic recombination as the means to transfer the desired character from the wild species into the cultivated plant. This paper deals with the cytogenetic behaviour of the trispecific hybrids and six of their first backcross progenies (BC1), in order to predict the chances of 'glandless-seed and glanded-plant' trait introgression.

Materials and Methods

Plant materials: Two trispecific hybrids have been created in the Department of Intertropical Crop Husbandry of the Faculty of Agricultural Sciences in Gembloux (Maréchal 1983). These hybrids include *G. hirsutum* L. 2 (A_hD_h) as recipient species, the Australian wild diploid *G. sturtianum* Will. ($2C_1$) as donor parent, and two American wild diploids *Gossypium thurberi* Tod. ($2D_1$) and *Gossypium raimondii* Ulbr. ($2D_5$) as bridge species. These hybrids are designated by the initials TSH for (*G. thurberi* × *G. sturtianum* × *G. hirsutum*; $A_hD_hD_1C_1$ genome) and HRS for (*G. hirsutum* × *G. raimondii* × *G. sturtianum*; $A_hD_hD_5C_1$ genome), respectively. Figure 1 shows the crossing schemes followed to obtain these genotypes. The meristems of the bispecific hybrids were treated with a 0.2% aqueous solution of colchicine for 24 h, as described by Beasley (1940), and tetraploid branches were selected and grafted on to vigorous plantlets of *G. hirsutum*. Both trispecific hybrids were backcrossed to three *G. hirsutum* glanded varieties (C2, NC8 and Stam F) originating from Africa and an American glandless cultivar (LPB5). The gland density of the hybrid seeds was evaluated under a stereomicroscope Wild M3Z (Leica, Herbrugg, Switzerland) according to a score scale ranging from 0 for completely glandless to 10 for fully glanded seeds. To obtain the BC1 analysed in this programme, it was necessary, immediately after pollination, to apply a solution of growth regulators (100 mg/l naphtoxyacetic acid, 50 mg/l gibberellic acid) to the ovary to cultivate the hybrid seeds *in vitro* on the medium of Stewart and Hsu (1977) and to graft some seedlings on to vigorous *G. hirsutum* seedlings (Vroh Bi 1994). All the plants used in the experiments were grown in a greenhouse in Gembloux, Belgium.

Cytological analysis: Meiotic studies were performed on flower buds fixed in Carnoy's solution (95% ethanol–chloroform–glacial acetic acid, 6:3:1 v:v:v) for 72 h and stored at 4°C in 70% ethanol until their evaluation. Microsporocytes were squashed and stained with 1.5% acetocarmine solution, and examined with a microscope Nf Jena (Carl Zeiss, Jena, Germany) under oil immersion. Meiotic configurations and chiasma counts were recorded for each genotype. Statistical comparisons were made by applying F-tests with Minitab statistical software, version 9.1 (Minitab Inc. 1992, State College, Evanston, IL, USA). A few anaphase I plates were inspected to assess the behaviour of univalents. For each plant, the development of pollen mother cells (PMCs) was examined from 1000 intact microsporocytes at the tetrad stage. About 1000 pollen grains were dipped in 1.5% acetocarmine solution for 30 min and pollen fertility was estimated as the percentage of stainable pollen; only large, bright red grains were considered fertile. In the analysis of the tetrad stage and pollen fertility, the parental species were used as controls.

Results

Seed production and gossypol gland evaluation

During the past four years, the trispecific hybrids were pollinated by *G. hirsutum*. On average, 1000 crosses produced only 104 BC1 seeds. Gland evaluation showed that six BC1 seeds were glandless (level 0), most of the seeds showed a reduced gossypol gland pattern (level 1–9) compared with the seeds of cultivated cotton, which are completely glanded (level 10). Intermediate gland patterns (level 4–7) were the most frequent (Mergeai et al. 1996). At present, 21 BC1 plants are growing in a greenhouse, but only six (for which analysis at metaphase I was possible) are reported in this study. The level of seed glands for TSH × C2/10, TSH × LPB5/4, HRS × LPB5/5, TSH × NC8/9, HRS × NC8/3 and TSH × NC8/5 were 5, 2, 4, 6, 2 and 0, respectively. Particular attention was paid to the glandless seed TSH × NC8/5 (Fig. 2A). This plant survived after *in vitro* culture and grafting. The density of gossypol glands on its leaves (Fig. 2B) was 292/cm^2. This 'glandless-seed and glanded-plant' (Fig. 2C) flowered abundantly in the greenhouse. Evaluation of the foliar gland density per cm^2 in the parental genotypes of this BC1 showed 315 in *G. sturtianum*, 598 in *G. thurberi*, 390 in the trispecific TSH hybrids and 240 in cultivated cotton *G. hirsutum* (variety NC8). The introgressed BC1, TSH × NC8/5 is perennial in growth habit.

Chromosome number and association at metaphase I (MI)

Cytogenetic data from both trispecific hybrids (TSH, HRS) and six of their BC1 are listed in Table 1. Univalents, bivalents and multivalents including tri-, tetra-, penta- and hexa-valents were observed. Chromosome counts showed that both trispecific hybrids have 52 chromosomes (Fig. 3A). Chromosome association frequency per cell was 15.07I + 15.34II + 0.93III + 0.69IV + 0.26VI in TSH and 14.42I + 17.03II + 0.82III + 0.15IV + 0.07VI in HRS (Table 1). Among the BC1, aneuploidy was observed in TSH × C2/10, TSH × LPB5/4, TSH × LPB5/5 with $2n = 4x + 2 = 54$, and in TSH × NC8/9 with $2n = 4x + 4 = 56$. The presence of these aneuploid plants

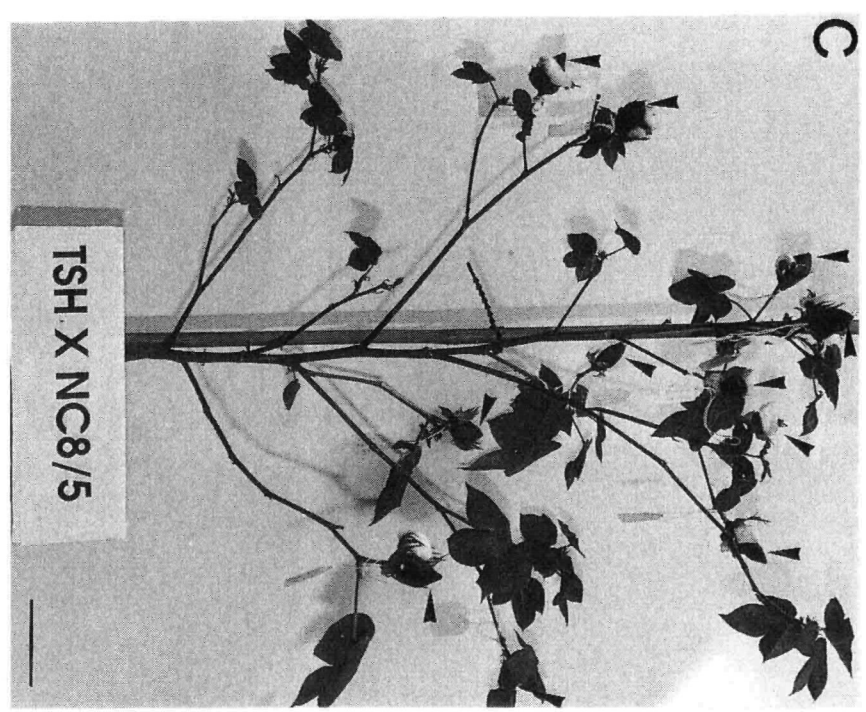

Fig. 2: The introgressed backcross 1 plant. A. The glandless seed of TSH × NC8/5 (scale bar = 0.5 mm). B. The glanded leaf of TSH × NC8/5 (scale bar = 2 mm). C. The 'glandless-seed and glanded-plant' TSH × NC8/5 obtained after *in vitro* culture of seed and plantlet grafting. The bolls are indicated by arrowheads (scale bar = 68 mm)

in the first backcross generation indicates that functional eggs from the trispecific hybrids containing 28 and 30 chromosomes instead of 26 were fertilized by normal pollen grains from *G. hirsutum*. The BC1 plants HRS × NC8/3 had 52 chromosomes. The 'glandless-seed and glanded-plant' type was also characterized by 52 chromosomes (Fig. 3B). This is probably the first report on a 'glandless-seed and glanded-plant' BC1 with 52 chromosomes. Its chromosome configuration at MI was 5.26I + 20.61II + 0.69III + 0.77IV. All the metaphase I plates observed in the trispecific hybrids and in the BC1 showed univalents and bivalents, while multivalents occurred with different frequencies; for example, the trispecific hybrids lacked pentavalents and no hexavalent was present in the BC1 plants. The number of univalents ranged from one in the 'glandless-seed and glanded-plant' TSH × NC8/5 to 24 in the trispecific hybrid TSH. At anaphase I, some of the univalents appeared as laggards (Fig. 3C). Laggards were more frequent in the trispecific hybrids than in the BC1. Although the average number of

Table 1: Mean frequencies of chromosome associations and chiasmata observed at meiotic metaphase I in the trispecific hybrids and their BC1; 26 pollen mother cells were observed in each genotype. The range of each configuration is given in parenthesis

Genotypes	No. of chromosomes	Chromosome associations						Mean of chiasmata
		I	II	III	IV	V	VI	
TSH	52	15.07 ± 0.78 (10–24)	15.34 ± 0.49 (11–20)	0.82 ± 0.17 (0–3)	0.46 ± 0.14 (0–2)		0.26 ± 0.1 (0–2)	37.30 ± 0.73 (32–45)
HRS	52	14.42 ± 0.76 (7–21)	17.03 ± 0.49 (12–21)	0.93 ± 0.19 (0–3)	0.15 ± 0.07 (0–1)		0.07 ± 0.05 (0–1)	36.88 ± 0.85 (31–46)
TSH × NC8/5	52	5.26 ± 0.52 (1–11)	20.61 ± 0.57 (12–24)	0.79 ± (0.17 (0–3)	0.77 ± 0.15 (0–3)			50.38 ± 0.63 (44–59)
TSH × NC8/9	56	7.42 ± 0.43 (3–11)	21.65 ± 0.39 (18–25)	1.34 ± 0.21 (0–4)	0.30 ± 0.12 (0–2)			39.57 ± 0.59 (32–47)
TSH × C2/10	54	13.15 ± 0.62 (10–26)	17.51 ± 0.58 (12–21)	1.61 ± 0.22 (0–4)	0.23 ± 0.08 (0–1)			36.53 ± 0.26 (32–40)
TSH × LPB5/4	54	8.96 ± 0.48 (6–13)	18.09 ± 0.49 (13–22)	2.61 ± 0.30 (0–5)	0.11 ± 0.06 (0–1)	0.1 ± 0.06 (0–1)		41.26 ± 0.16 (40–43)
HRS × NC8/3	52	8.26 ± 0.41 (3–12)	19.65 ± 0.39 (17–22)	1.09 ± 0.20 (0–3)	0.30 ± 0.10 (0–2)			39.38 ± 0.45 (32–46)
HRS × LPB5/5	54	6.92 ± 0.52 (2–11)	21.30 ± 0.41 (18–26)	1.11 ± 0.19 (0–3)	0.29 ± 0.06 (0–1)			41.61 ± 0.09 (41–42)

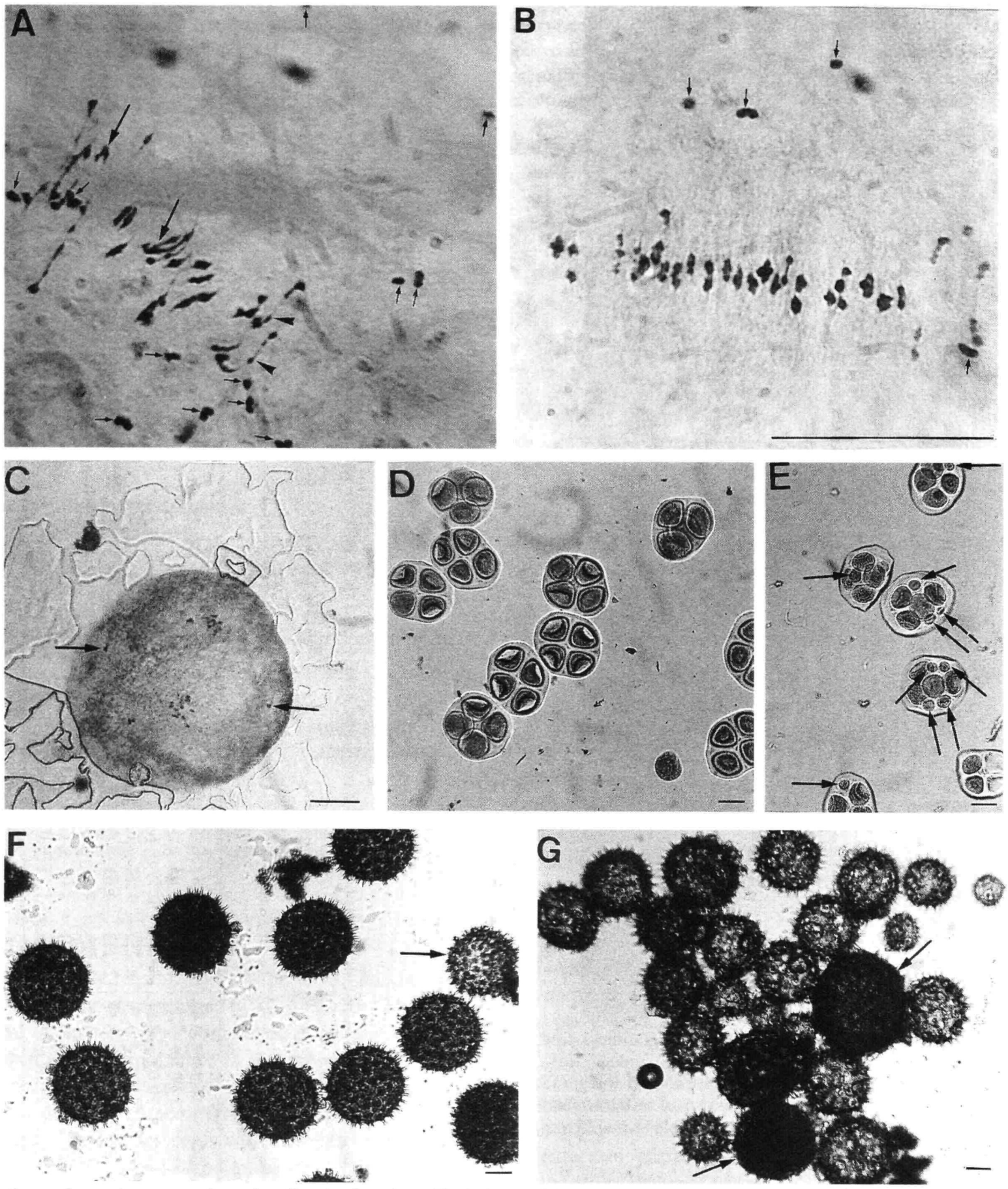

Fig. 3: Meiotic configurations, tetrads and pollen in the trispecific hybrid TSH (*G. thurberi* × *G. sturtianum* × *G. hirsutum*), the 'glandless-seed and glanded-plant' TSH × NC8/5 and the cultivated cotton *G. hirsutum*. A. Meiotic metaphase I cell showing 12 univalents (small arrows), 13 bivalents, two trivalents (arrow heads) and two tetravalents (large arrows) in TSH. B. Meiotic metaphase I cell showing four univalents (arrowed) and 24 bivalents in TSH × NC8/5. C. Anaphase I cell showing lagging chromosomes (arrowed) in TSH × NC8/5. D. Tetrads in *G. hirsutum*. E. Tetrads with micronuclei (arrowed) in TSH. F. Pollen grains in *G. hirsutum*; stainless grain is arrowed, note the uniform size of pollen grains. G. Pollen grains in TSH; stained pollen grains are arrowed, note variation in pollen grain size. Scale bars = 10 μm

univalents in TSH (15.07) was slightly higher than that of HRS (14.42), the difference was not significant. The mean numbers of univalents in the trispecific hybrids were significantly higher than in the six BC1 plants. Among the plants analysed, TSH × NC8/5 expressing the 'glandless-seed and glanded-plant' trait also showed the lowest frequencies of univalents (5.26), indicating that at least 46 chromosomes of this plant are associated in bivalent and multivalent structures. Despite the small size of the cotton chromosomes, it is possible to identify the genomic origin of some chromosomes at MI. The chromosomes of the A, D and C genomes are small, medium and large, respectively. In the MI cells of the BC1 analysed, the three types of chromosomes were observed. This showed that all the BC1 had the same genomic constitution ($A_hC_1D_hD_1$ or $A_hC_1D_hD_5$) as the trispecific hybrids, with the largest univalents probably belonging to the C1 donor genome. The range and the mean numbers of bivalents observed are given in Table 1. At the P = 0.05 level, the difference between the mean number of bivalent in TSH (15.34) and HRS (17.03) was significant. In the 'glandless-seed and glanded-plant' TSH × NC8/5, the number of bivalents ranged from 12 to 24, with the most frequent number being 22. The average number of bivalents in

Table 2: Analysis of tetrads and pollen fertility in the trispecific hybrids and the BC1

Genotypes	Microsporocytes				Pollen fertility	
	Diads	Triads	Tetrads with micronuclei	Normal tetrads	Total no. of pollens	No. of stainable pollens
TSH	0	16	822	162	1036	30 (2.90%)
HRS	0	12	864	124	1003	90 (8.97%)
TSH × NC8/5	0	22	376	602	1177	360 (30.59%)
TSH × NC8/9	4	10	426	560	1000	150 (15.00%)
TSH × C2/10	12	181	709	98	1005	0 (0.0%)
TSH × LPB5/4	0	9	539	452	1062	219 (20.62%)
HRS × NC8/3	2	3	318	677	1157	466 (40.28%)
HRS × LPB5/5	1	8	479	512	1070	330 (30.84%)

this genotype was 20.61, which is significantly higher ($P = 0.01$) than the average number (15.34) observed in the trispecific hybrid TSH used as its female parent. Multivalents in the trispecific hybrids ranged from trivalents to hexavalents, the most frequent being trivalents. The range of multivalents was reduced in the BC1, in which no hexavalents were observed. The 'glandless-seed and glanded-plant' BC1 lacked penta- and hexavalents. Various shapes of bivalents and multivalents were observed, but the genomic origin of their chromosomes could not be identified easily. Heterogenetic pairing in these plants would enable exchanges of genetic material between the genomes involved in the crosses.

Chiasma frequency

The level of exchange was assessed by chiasma counts at MI (Table 1). There was no significant difference between the average numbers of both trispecific hybrids (37.30 in TSH and 36.88 in HRS), but the mean number of chiasmata (50.38), in the 'glandless-seed and glanded-plant' TSH × NC8/5 was significantly higher at the $P = 0.001$ level than that of any other plant under study. Most bivalents had two chiasmata, but bivalents with three chiasmata were also present. Terminal chiasmata were easily scored, while interstitial chiasmata were difficult to identify owing to the relatively small size of cotton chromosomes. Similar observations were reported by Srivastava (1980a).

Analysis of tetrads and pollen fertility

Data from the development of PMCs are presented in Table 2. All the tetrads observed in the cultivated species, *G. hirsutum*, were normal (Fig. 3D), while the trispecific hybrids and the BC1 showed triads, normal tetrads and abnormal tetrads containing micronuclei (Fig. 3E). Occasional microsporocytes with six or eight cells of equal size (multicells) were observed in the trispecific hybrids. On average, the number of micronuclei per tetrad ranged from one to four in the trispecific hybrids and from one to three in the BC1. The micronuclei probably originated from laggards at anaphase I. The micronuclei differed in size, according to the number of chromosomes or fragments incorporated. In the 'glandless-seed and glanded-plant' TSH × NC8/5, 2.20% of the sporocytes were triads, 60.20% were normal tetrads without micronuclei, and 37.60% were abnormal tetrads containing micronuclei. The number of abnormal tetrads in HRS (864) was slightly higher than that observed in TSH (822), although the latter showed a slightly higher number of univalents compared with HRS. This may be because most univalents in TSH were incorporated in the nuclei owing to their favourable positioning before meiotic divisions.

The pollen fertility (Table 2) of the parental species, *G. hirsutum*, *G. raimondii*, *G. thurberi*, and *G. sturtianum* was 98.69%, 96.10%, 88.64% and 98.67%, respectively. In each of these species, the pollen grain size was mostly uniform (Fig. 3F). In the trispecific hybrids and the BC1, pollen fertility proved difficult to evaluate since pollen grain size was highly variable (Fig. 3G). In the BC1, pollen fertility ranged from 0% for TSH × C2/10 to 40.28% for HRS × NC8/3. In the 'glandless-seed and glanded-plant' BC1, we observed 30.59% fertile pollen grains, which is tenfold higher than the pollen fertility of its female parent TSH, but threefold lower than the pollen fertility of the pollinator parent *G. hirsutum*.

Discussion

Previous studies using bispecific crosses to transfer the seed gland repression factors from the Australian wild diploids into the tetraploid cultivated cotton indicated constraints due to chromosome addition (Altman et al. 1987, Dilday 1986, Koto 1989, Rooney et al. 1991). A prospective method of introgressing the trait is to confront the A_h and D_h subgenomes of *G. hirsutum* with the whole genome of a diploid Australian species in a genomic background favouring recombination. To date, only Shuijing and Biling (1993) have adopted such a strategy by developing the trispecific hybrid *G. arboreum* × *G. bickii* × *G. hirsutum* ($A_hA_2D_hG_1$), with $2n = 4x = 52$ chromosomes. The MI configuration reported by these authors was 41.04I + 4.54II + 0.57III + 0.04IV in the trispecific hybrid. Some of the cells showed only univalents. In this crossing scheme, the A_h chromosomes of *G. hirsutum* carrying the Gl_2 allele will pair mostly with their homologous A_2 chromosomes of *G. arboreum*. The large G_1 chromosomes of *G. bickii*, which have the seed gland repression factors and the small D_h chromosomes of *G. hirsutum* containing the Gl_3 allele, will remain unassociated because of their size difference and the lack of translocation. The low frequency of chromosome associations observed in this trispecific hybrid agrees with the pairing affinities between the genomes involved. Indeed, the main force which controls regular pairing behaviour in *Gossypium* is thought to be the difference in the degree of chromosome condensation (Endrizzi 1962). Homoeologous chromosomes of differently sized *Gossypium* genomes rarely pair. The crossing schemes developed in the present study were based on: (1) the translocations that occurred between A, D and C during *Gossypium* species divergence; (2) the higher pairing affinity of

the donor C chromosomes (large size) for A chromosomes (medium size) than for D chromosomes (small size) (Endrizzi et al. 1985); and (3) the greater efficiency of the seed gossypol gland repression mechanism in the wild Australian species against the A genome than the D genome (Mergeai 1992).

The univalents observed in this study can be attributed to asynapsis because of the lack of homology between the different sets of chromosomes, or to the failure of the chromosomes to remain associated (desynapsis). Asynapsis and/or desynapsis are therefore more frequent in the trispecific hybrids than in the BC1. The presence of laggards demonstrated the occurrence of meiotic disturbances leading to chromosome restitution.

Apart from the nature of the bridge species (*G. thurberi* in TSH and *G. raimondii* in HRS), the main difference between the trispecific hybrids is the last step of the crossing schemes: two tetraploid genotypes were crossed (4x × 4x) to obtain TSH, while HRS was derived from a cross between a hexaploid and a diploid genotype (6x × 2x). Brown and Menzel (1950) and Louant and Maréchal (1975) found no significant difference between chromosome pairing behaviour of cotton trispecific hybrids obtained by crossing 4x × 4x and 6x × 2x. The difference in bivalent frequency observed between TSH and HRS is most likely due to pairing affinities of the bridge species chromosomes for the D subgenome of the cultivated cotton *G. hirsutum* $2(AD)_1$. Indeed, of the two bridge species used, the chromosomes of *G. raimondii* pair more frequently with *G. hirsutum* chromosomes (Geever et al. 1989, N'dungo et al. 1989). During *Gossypium* evolution, segmental interchanges took place between the A, C and D genomes (Maréchal 1974). Hence, when they are present in the hybrid, the residual homology between the three sets of chromosomes can lead to multivalents. The formation of multivalents in trispecific hybrids can facilitate exchanges. However, the decrease of multivalents in the introgressed BC1 is highly desirable, since complex multivalents can interfere with fertility because of non-disjunction.

Comparison of the mean numbers of chiasmata suggests that the highest frequency of exchange occurred in the BC1 expressing the 'glandless-seed and glanded-plant' trait. Considering that in MI the terminalization of chiasmata reaches its maximum, the actual frequency of chiasmata occurring in this plant may be even higher than observed. An increase in chiasma frequency improves meiotic regularity because chiasmata determine proper orientation and thus distribution of chromosomes at anaphase (Srivastava 1980b, Sybenga 1992). Chiasmata also represent recombination leading to genetic diversity in gametes (Kumar et al. 1990). Therefore, the high frequency of chiasmata observed in TSH × NC8/5 ($A_hC_1D_hD_1$) opens interesting possibilities for the introgression of the 'glandless-seed and glanded-plant' trait into commercial cultivars of cotton. In such combinations, D_1 chromosomes of the bridge species pair mostly with their homologous D_h chromosomes of *G. hirsutum*, while C1 chromosomes, which have the seed gossypol glands repression mechanism, are confronted mostly with the A_h subgenome that carries the Gl_2 allele determining seed gossypol glands. These results suggest that introgression of the 'glandless-seed and glanded-plant' from *G. sturtianum* into a tetraploid cotton is feasible. Despite the high chiasma frequency, it is impossible to determine whether the expression of the 'glandless-seed and glanded-plant' trait in the BC1 results from recombinations or the presence of univalent C_1 chromosomes carrying the seed gossypol glands repression factors.

We grafted the introgressed BC1 plant on to seven vigorous plantlets of *G. hirsutum*. These plants are now involved in a recurrent backcrossing programme. However, a more accurate evaluation of the chromosomes involved in meiotic configurations is essential to understand the events leading to the expression of the desired trait in a tetraploid cotton genotype. To achieve this objective, investigations have been undertaken on the DNA level (Vroh Bi et al. 1996). In particular, genomic *in situ* hybridization and RFLP analysis are under way to identify the chromosomes of the introgressed BC1 plant.

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