Euphytica **106:** 243–251, 1999.

Development of high-gossypol cotton plants with low-gossypol seeds using trispecies bridge crosses and *in vitro* culture of seed embryos

I. Vroh Bi[1], J.P. Baudoin[1], B. Hau[2] & G. Mergeai[1]
[1] *Unité de Phytotechnie des Régions Intertropicales, Faculté Universitaire des Sciences Agronomiques, 2 Passage des Déportés, B-5030 Gembloux, Belgium*
[2] *Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD). B.P. 5035, 34032 Montpellier Cedex, France*

Received 6 January 1998; accepted 18 November 1998

Key words: Cotton, gossypol glands, wide crosses, hybrid rescue

Summary

The objective of this work is to develop an upland cotton, *Gossypium hirsutum* L. [2n = 4x = 52, 2(AD)$_h$], having a reduced level of gossypol in the seeds for food and feed uses, and a high level of gossypol in the remaining organs to limit pest incidence. Using *G. sturtianum* Willis (2n = 2x = 26, 2C$_1$) as donor and *G. thurberi* Torado (2n = 2x = 26, 2D$_1$) or *G. raimondii* Ulbrich (2n = 2x = 26, 2D$_5$) as bridge species, two trispecies hybrids *G. thurberi–G. sturtianum–G. hirsutum* and *G. hirsutum–G. raimondii– G. sturtianum* were synthesized. Both trispecies hybrids were male sterile. Recurrent backcrossing to *G. hirsutum* as pollinator and selfing of the second backcross (BC) progenies resulted in seeds which were rescued by *in vitro* culture. In total, 1208 flowers of the trispecies hybrids and their BC progenies yielded 192 seed embryos from which 62 plants were obtained. Cytogenetic analyses indicated a relatively high frequency of chromosome pairing and chiasmata. The gland levels in backcross seeds ranged from glandless seeds to normally glanded seeds. All vegetative parts of those hybrids were glanded, but a wide range of variability for gland density was observed on leaf, stem, bract and calyx. Plants derived from seeds having a reduced level of gossypol constitute very interesting germplasm to develop a cultivated glanded cotton with low-gossypol seeds.

Cotton (*Gossypium* spp.) is not only the leading textile crop, it is also an important food crop. Cottonseed contains approximately 35 to 38% oil and 35 to 39% protein (Marquié, 1987). Research in animal and human nutrition showed that cottonseed is a source of high quality protein (Lusas & Jividen, 1987; Marquié, 1987; Zhuge et al., 1988; Alford et al., 1996). However, the development of edible protein products from cottonseed is limited by the presence of a toxic substance, gossypol. This polyphenolic compound occurs in the pigment glands of cotton. Bound gossypol is toxic to monogastric animals including humans, and also causes discoloration in foods (Forman et al., 1991).

Two different approaches have emerged to eliminate or minimize adverse effects of gossypol in cottonseed. The first is the development of chemical, mechanical and thermic processes that remove or deactivate gossypol from the seed derivatives (Vix et al., 1971; Kadan et al., 1979; Rahma & Narasinga, 1984; Liadakis et al., 1993; Hron et al., 1996). Although these procedures are efficient, they increase the oil extraction cost and decrease the nutritional value of cottonseed products by reducing bioavailable lysine (Fisher et al., 1988). The second approach is the development of glandless varieties of cotton that are totally free of pigment glands. The formation of gossypol glands in the tetraploid cultivated cotton, *G. hirsutum* L. [2n = 4x = 52, 2(AD)$_h$] is controlled by two main alleles Gl_2 and Gl_3. McMichael (1954) reported two mutant recessive alleles gl_2 and gl_3 which completely remove pigment glands from the whole plant, and give nontoxic cottonseed. However, completely glandless cotton are sometimes attacked by various insects that

do not normally feed on glanded cottons (Stipanovic et al., 1975; Bell et al., 1978; Garas & Waiss, 1986; Altman et al., 1990; Khoshkhoo et al., 1994; Calhoun, 1997). The ideal cultivated cotton should have a reduced level of gossypol in the seed for optimal food and feed uses, and a high level of gossypol in the remaining organs to limit pest incidence. Among the 50 species of *Gossypium*, the glandless-seed and glanded-plant trait exists only in some Australian wild diploid species belonging to *Sturtia* and *Hibiscoidea* sections (Brubaker et al., 1996). These Australian cottons are phylogenetically the most distant from the upland cotton (Fryxell et al., 1992). We employed bridge crosses and recurrent backcrossing to attempt the introgression of seed gland elimination factor(s) from *G. sturtianum* Willis (2n = 2x = 26, 2C1) into *G. hirsutum*. The present paper reports the results of production, seed rescue, gossypol gland behaviour and cytological analysis of the hybrid plants.

Materials and methods

Plant materials

Plants consisted of two trispecific hybrids containing *G. hirsutum* $2(AD)_h$ as recipient species, *G. sturtianum* ($2C_1$) as donor parent, and two American wild diploids *G. thurberi* Torado ($2D_1$) and *G. raimondii* Ulbrich ($2D_5$) as bridge species. These hybrids are respectively designated by TSH for [(*G. thurberi* x*G. sturtianum*) doubled × *G. hirsutum*; $A_hC_1D_hD_1$] and HRS for [(*G. hirsutum* × *G. raimondii*) doubled × *G. sturtianum*; $A_hC_1D_hD_5$]. The intermediate bispecific hybrids were doubled by colchiploidization as described in Beasley (1940), the schemes to create the trispecies hybrids are detailed in Vroh Bi et al. (1997). Both trispecies hybrids were backcrossed in greenhouse with three *G. hirsutum* glanded varieties (C2, NC8 and Stam F). The resulting first backcross (BC1) plants were crossed using *G. hirsutum* (var. Stam F) as pollinator to produce second backcross (BC2) plants. The BC2 were either crossed with *G. hirsutum* (var. Stam F) or manually self-pollinated to obtain the third backcross (BC3) plants or the BC2 selfed progeny (BC2sp). After each cross, a distilled water solution of growth regulators (100 mg L^{-1} naphtoxyacetic acid + 50 mg L^{-1} gibberellic acid) was applied on the ovary to prevent boll shedding.

In vitro *culture and gland evaluation*

Seed gland levels were assessed under a stereomicroscope just before *in vitro* culture in a fume hood. The seeds were desinfected by immersion in ethanol 70% for 1 min, and in 1.5% calcium hypochlorite solution for 5 min, followed by three rinses in sterile deionized water. Seeds were aseptically dissected in petri dishes to remove maternal teguments. After soaking the embryos in sterile deionized water for 1 h to facilitate the removal of the testa, gland density of naked embryos was assessed under a binocular microscope according to a visual scale ranging from 0 for totally glandless embryo to 10 which is the gland level in normally glanded cultivated cottonseed. Seed embryos were cultured on the medium of Stewart and Hsu (1977) in a growth chamber regulated at 27 °C, with 12 h photoperiod (100 μEinstein m^{-2} s^{-1}). When the root system was sufficiently developed, seedlings were placed in a soil mix of peat:compost:sand (1:1:1) and transferred in a growth room for acclimatization. Seedlings were covered with a perforated plastic cups and incubated at 28 °C/24 °C day/night temperature, with 12 h photoperiod (380 μEinstein m^{-2} s^{-1}) and 60 to 70% relative air humidity. The plastic cups were removed a few days later according to the vigor of seedlings. During acclimatization period, each plantlet was irrigated once a week with 500 mL of a nutritive solution composed of the following elements (mg L^{-1}): 900 $Ca(NO_3)_2.4H_2O$; 590 mg$SO_4.7H_2O$; 180 K_2SO_4; 390 KNO_3; 200 KH_2PO_4; 11.5 K_2HPO_4; 15 000 $MnCl_2.4H_2O$; 10 000 $ZnSO_4.7H_2O$; 10 000 H_3BO_3; 2 000 $CuSO_4.5H_2O$; 35 000 $FeSO_4.7H_2O$ and a few drops of H_2S0_4. The surviving plantlets were then transferred in greenhouse where grafting on vigorous *G. hirsutum* plantlet was carried out if necessary.

We collected from 44 mature plants 10 terminal leaves (2.5 cm wide), 10 pieces of stems, five flower buds of 3 mm diameter (two counts per calyx) and 10 bracts of the collected buds. Number of glands on leaves and stems was determined per cm^2 while on flower buds and bracts, counts were made per mm^2 and extrapollated to 1 cm^2. Data were analyzed with One-way analysis of variance (AOVONEWAY) and Pearson's correlation coefficients using Minitab software version 10.1 (Minitab Inc. 1994 State College, U.S.A.).

Cytogenetic studies

Flower buds were fixed in Carnoy's (6:3:1, chloroform: ethanol:glacial acetic acid) for 48 h, and stored

in 70% ethanol at 4 °C until use. The meiotic behaviour of both trispecific hybrids, four BC1, three BC2, three BC3 and one BC2 selfed progeny (BC2sp) was analyzed. For each plant 56 metaphase-I plates, 1 000 sporocytes at tetrad stage, and 1 000 pollen grains were observed using squashes and staining in 1.5% acetocarmine. The plants studied cytologically are those on which pollen mother cells (PMCs) were available at metaphase I stage. Cytogenetic data were also subjected to AOVONEWAY on Minitab software.

Results

Production of the backcross derivatives and gland behaviour

Hybridization data of trispecies hybrids and backcross derivatives are given in Table 1. Seed set in these crosses was very low. For instance, backcrosses of the trispecies hybrids with *G. hirsutum* set only one seed per 10 bolls in HRS and 2 seeds per 10 bolls in HRS. Seed set increased with increased backcrosses but remained significantly lower than in cultivated cotton. Indeed, under the same greenhouse conditions, 10 bolls of the cultivated cotton *G. hirsutum* variety StamF yielded on average 240 seeds. Among 24 BC1 pollinated with *G. hirsutum* (Table 1), only two plants gave seeds, showing that the majority of the BC1 plants are female sterile. In the BC2 generation, two out of three plants set seeds. Fruit abscission represented only 4.4% of the total number of pollinated flowers in trispecies hybrids and backross progenies, due certainly to the application of growth regulators. The mean number of motes (aborted seeds) per mature boll of trispecies hybrids, BC1 and BC2 plants was 15, 10 and 7, respectively. Scarcity of seeds, empty seeds (Table 1) and motes are commonly found in difficult crosses. The gossypol glanding pattern in the seeds of backcross progenies ranged from 0 to 10 (Table 2), and seed embryos varied in morphology and size (Figure 1). After germination, glands of seedlings had a light color. As chlorophyl formed and the cotyledons and stem became green, the glands became darker and increased in number. In total, fifty seven percent of the seeds germinated *in vitro* (Table 1) but some seedlings did not grew further, because the cotyledons failed to open and/or the radicule tip became necrotic. Some well-established seedlings died during acclimatization when their roots stopped growing or when new leaves failed to emerge. Nevertheless, some BC2

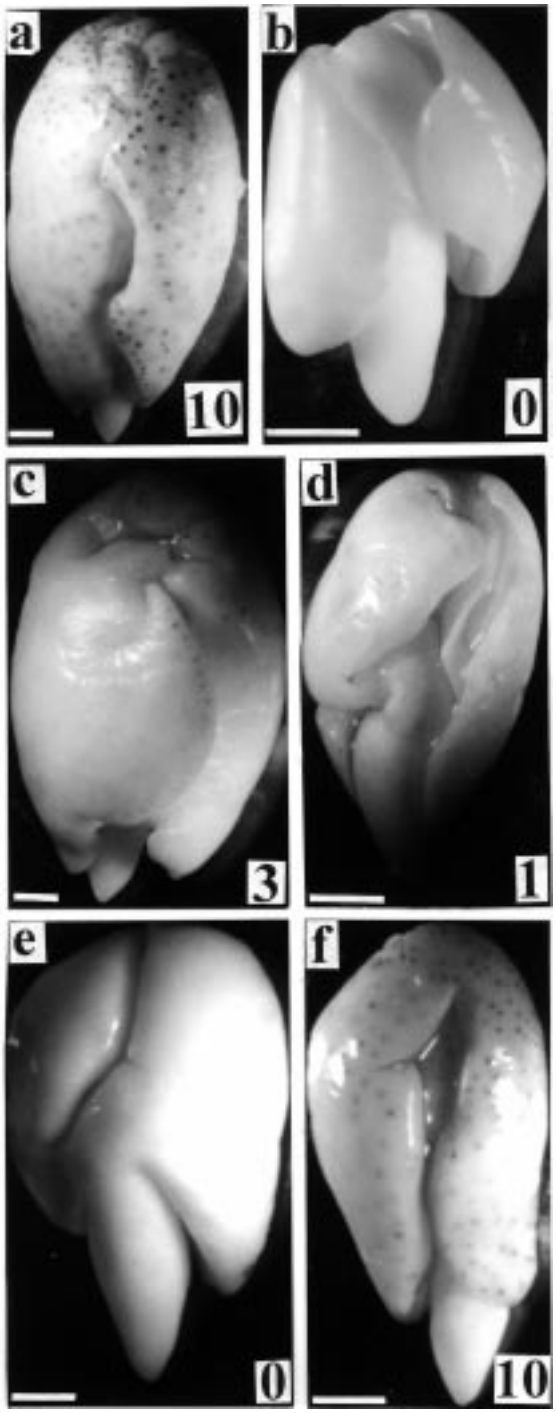

Figure 1a–f. Gossypol glanding patterns in the seeds of *G. hirsutum, G. sturtianum* and backcross progenies. (a) *G. hirsutum* StamF (a normally glanded variety). (b) *G. sturtianum* Willis (glandless seed). (c, d) Two backcross 1(low-gossypol seed). (e) A backcross 2 (low-gossypol seed). (f) A backcross 2 (normally glanded seed). Numbers 0, 1, 3 and 10 refer to the seed gland level. Scale bar = 0.5 mm.

Table 1. Production details of backcross progenies from the trispecies hybrids TSH and HRS

Cross	N° of plants pollinated	N° of flowers pollinated	N° of mature bolls	seed set	N° of empty seeds	N° of seeds cultivated *in vitro*	N° and % of seeds germinated *in vitro*	N° of plants after hardening
HRS × *G. hirs*[a]	9	615	592	55	2	53	18 (33.9%)	17
TSH × *G. hirs*	8	395	383	74	11	63	42 (66.6%)	14
Backcross 1 × *G. hirs*	24	110	95	23	1	22	11 (50.0%)	6
Backcross 2 × *G. hirs*	3	4	4	22	3	19	19 (100%)	16
Backcross 2 selfed	3	29	28	18	0	18	18 (100%)	9
Total	47	1153	1102	192	17	175	108 (61.7%)	62

[a] *G. hirsutum* used as male.

Table 2. Distribution of seeds number per gland level in the backcross generations

N° of seeds in each gland level per generation						
Seed gland level	BC1[a] (HRS)	BC1[b] (TSH)	BC2	BC3	BC2sp	Total N° of seeds per gland level
0	2	5	2	0	0	9
1	6	1	0	0	2	9
2	6	5	1	1	4	17
3	7	8	2	3	5	25
4	5	8	4	5	2	24
5	11	7	6	3	1	28
6	4	8	3	3	0	18
7	5	12	4	1	0	22
8	3	3	0	2	0	8
9	3	2	0	1	1	7
10	1	4	0	0	3	8
Total	53	63	22	19	18	–

[a] BC1 derived from trispecies hybrid HRS
[b] BC1 derived from trispecies hybrid TSH

and BC3 plantlets displayed hybrid vigor and grew faster than cultivated cotton used as control. Sixty two plants (Table 1), which were successfully rescued are presently maintained in greenhouse, where grafting on vigorous cultivated cotton plant is often used to rescue unbalanced plants. All the plants produced in this study were glanded but the gossypol gland density varied between mature plants and organs (Table 3). An example of variation in gland distribution and gland shape is given in Figure 2. Non significant correlation was found between seed gland level and gland density on leaf ($r = -0.024$), stem ($r = -0.178$), bract ($r = -0.101$) and calyx ($r = 0.167$). Positive and significant associations ($P = 0.05$) were established between the gland density on leaf and bract ($r = 0.353$), bract and calyx ($r = 0.348$). Morphologically, both trispecies hybrids were intermediate between their respective parents. The BC1 plants contained a wide range of plant types and seed size. In the BC2, BC2sp and BC3 hybrids, the pubescence, leaf shape and flower colour were closer to *G. hirsutum* than to the wild species. The boll size was generally small and lint production was considerably lower than in *G. hirsutum*. All the hybrids plants produced were perennial in growth habit.

Cytogenetic analysis

Details of meiotic studies are presented in Table 4. Both trispecies hybrids showed 52 chromosomes as in tetraploid cotton and aneuploidy were observed

Table 3. Evaluation of glands in seeds and plants of *G. hirsutum*, *G. sturtianum*, trispecies hybrids and backcross progenies (Only plants for which cytological analysis was possible are reported)

Genotypes	Seed gland level	Mean number of gossypol glands per cm^2 of organs			
		Stems	Leaves	Bracts	Calyx
G. hirsutum	10	246 ± 6	1190 ± 10	154 ± 12	999 ± 19
G. sturtianum	0	94 ± 6	1492 ± 10	1284 ± 17	1868 ± 16
HRS	–	512 ± 9	1056 ± 20	1015 ± 31	1634 ± 9
TSH	–	306 ± 12	2379 ± 20	1141 ± 31	1700 ± 26
TSH × NC8/6[a,b]	5	207 ± 23	787 ± 3	1055 ± 36	1025 ± 9
TSH × NC8/7[b]	3	234 ± 34	776 ± 22	733 ± 30	881 ± 17
HRS × C2 (S1)[b]	5	495 ± 6	2484 ± 23	885 ± 13	1552 ± 10
HRS × C2/11[b]	2	486 ± 12	1002 ± 47	452 ± 12	910 ± 14
S1 × StamF/1 (BC2/1)[c]	4	417 ± 15	699 ± 15	152 ± 18	642 ± 9
S1 × StamF/2 (BC2/2)[c]	3	391 ± 27	2142 ± 27	298 ± 23	752 ± 9
S1 × StamF/3 (BC2/3)[c]	5	373 ± 5	692 ± 14	201 ± 4	1004 ± 19
BC2/1 × StamF/4[d]	4	350 ± 10	979 ± 26	153 ± 23	505 ± 24
BC2/1 × StamF/12[d]	5	210 ± 10	1102 ± 18	198 ± 13	1041 ± 37
BC2/3 × StamF/3[d]	5	818 ± 15	821 ± 20	154 ± 12	1101 ± 39
BC2/1sp/13[e]	4	458 ± 9	903 ± 10	155 ± 5	751 ± 21

– seed glands not evaluated
[a] TSH × NC8/6 = plant N° 6 obtained by pollinating TSH with *G. hirsutum* (variety NC8)
[b] Backcross 1, [c] Backcross 2, [d] Backcross 3, [e] selfed progeny derived from BC2/1

among the backcross progenies, (Figure 3a). Uni-, bi- and trivalents were present in all the PMCs (pollen mother cells) analyzed while tetravalents were lacking in some backcross plants, and hexavalents were only present in the trispecies hybrids (Table 4). The mean number of univalents of the backcross progenies are significantly lower than that of their respective trispecies parent. Conversely, the average number of bivalents in the backcross progenies are higher than that found in the trispecies hybrids. All the meiocytes exhibited ring and rod bivalents but the majority of the bivalents were ring, except in TSH which exhibited more rod bivalents, and in HRS where the mean numbers of rod and ring bivalents were equal. No significant differences were found between the mean number of chiasmata of the two trispecies hybrids. All the backcross plants (except in TSH × NC8/7) had significantly higher frequencies of chiasmata than in TSH and HRS. In general, rod bivalents had a single chiasma and ring bivalents had 2 chiasmata, although ring bivalents with more than 2 chiasmata were observed. Some chromosomes involved in heteromorphic configurations can not be assigned definitely to their respective genome because of their small size. Nevertheless, chromosomes of genome C are larger than those of A and D genome. The C_1 chromosomes of *G. sturtianum* are thus recognized when they are univalents (Figure 3a). Meiotic abnormalities observed were lagging chomosomes, unequal separation, tetrads with less or more than four nuclei (Figure 3b), and micropollen leading to male sterility (Figure 3c). The pollen fertility reached a maximum of 63.2% and 79.6% respectively in BC2 and BC3 plants (Table 4).

Discussion

Many of the world's hungry are located in warm climates where cotton is cultivated. Although cottonseed can contribute to solve the problem of hunger, its utilization in human nutrition is still limited by the presence of gossypol. Thus, a reduction in seed gossypol level is desirable. According to the regulations of the U.S. Food and Drug Administration (FDA), free gossypol content of edible cottonseed products should not exceed 450 ppm. The protein advisory group of the United Nations Food and Agriculture and World Health Organizations (FAO/WHO) set maximum value of 600 ppm (Lusas & Jividen, 1987). Since the use of different processes to eliminate or reduce gossypol failed, the most prospective way to reduce gossypol level from cottonseed is genetic research. Because the association of gossypol gland density with cotton plant resistance was demonstrated

Table 4. Microsporogenesis in the trispecies hybrids TSH, HRS and their backcross derivatives

Genotypes	N° of Chrs.[a]	Mean and range of chromosome configurations at metaphase I							XMT[b]	Tetrad stage		% of fertile pollen
		I	II			III	IV	VI		normal tetrads	abnormal tetrads	
			Ring	Rod	Total							
TSH	52	14.55 (7–21)	6.76 (2–12)	8.92 (3–16)	15.68 (11–21)	0.91 (0–3)	0.35 (0–1)	0.25 (0–2)	33.25 (16–46)	162	838	2.90
HRS	52	13.64 (9–24)	8.87 (4–16)	8.17 (4–14)	17.04 (12–21)	0.85 (0–3)	0.35 (0–2)	0.07 (0–1)	34.35 (28–46)	124	876	8.90
TSH × NC8/6	52	3.96 (2–6)	17.25 (16–19)	6.00 (3–8)	23.25*** (20–25)	0.32 (0–1)	0.10 (0–1)		40.17*** (34–49)	640	360	6.40
TSH × NC8/7	49	9.67 (7–13)	15.50 (13–19)	3.89 (1–7)	19.39*** (18–21)	0.17 (0–1)			34.80 (26–40)	650	350	2.00
HRS × C2 (S1)	53	6.45 (3–11)	18.10 (14–21)	4.46 (2–7)	22.56*** (19–25)	0.30 (0–3)	0.10 (0–1)		42.76*** (35–51)	808	192	9.50
HRS × C2/11	52	8.40 (0–11)	15.43 (13–18)	5.36 (2–9)	20.79*** (17–24)	0.67 (0–3)			41.78*** (36–52)	519	481	5.70
S1 × StamF/1 (BC2/1)	52	3.83 (1–9)	15.35 (11–21)	8.26 (3–12)	23.61*** (20–25)	0.31 (0–1)			42.12*** (34–50)	820	180	60.50
S1 × StamF/2 (BC2/2)	45	2.98 (0–5)	14.39 (11–16)	6.37 (4–10)	20.76*** (20–21)	0.16 (0–1)			48.67*** (42–58)	304	696	5.50
S1 × StamF/3 (BC2/3)	52	5.52 (2–8)	19.17 (15–22)	3.96 (1–7)	23.13*** (21–25)	0.07 (0–1)			38.96*** (35–46)	908	92	63.20
BC2/1 × StamF/4	54	3.01 (0–4)	18.09 (16–21)	7.21 (5–12)	25.30*** (24–27)	0.14 (0–1)			46.03*** (42–49)	689	311	54.40
BC2/1 × StamF/12	54	2.00 (0–6)	21.18 (18–25)	3.42 (0–6)	24.60*** (21–27)	0.23 (0–1)	0.51 (0–1)		48.26*** (44–56)	252	748	79.60
BC2/3 × StamF/3	54	2.08 (1–4)	20.26 (15–23)	4.46 (2–7)	24.72*** (22–26)	0.82 (0–2)			47.62*** (39–53)	711	289	46.40
BC2/1sp/13	52	1.71 (0–3)	18.65 (15–23)	6.07 (3–9)	24.72*** (23–26)	0.21 (0–2)	0.07 (0–1)		47.05*** (43–50)	885	115	11.40

[a] Number of chromosomes
[b] Mean number of chiasmata
*** $P < 0.001$, significantly higher than in the trispecies hybrid they derived from

also (Parrott et al., 1989; Hedin et al., 1992; Mohan et al., 1994; Rajarajeswari & Subbarao, 1997), cotton cultivar meeting both agricultural and seed processing needs should have a reduced level of gossypol in the seed and a high level of gossypol in the vegetative parts. The exploitation of the Australian wild diploid species having the glandless-seed and glanded-plant trait in breeding programs offers a possibility to cotton breeders. However, the introgression of desirable traits from the numerous diploid wild species of the *Gossypium* genus, into the main cultivated cotton *G. hirsutum* is not easy since this species is tetraploid. In this study, incompatibility barriers were expressed by the occurrence of embryo abortion resulting to motes and empty seeds. We have now undertaken the *in vitro* culture of very young embryos (Gill & Bajaj, 1984), to improve the recocery of hybrid plants in the BC1 generation. Nevertheless, this program allowed us to get some viable and vigorous plants with very interesting characteristics.

In such plants, the amount of segregation should be related to the extent of homogenetic and heterogenetic pairing. If pairing is completely homogenetic, only bivalents are formed from homologues and there would be no segregation of genes differenciating the parental species. However, if pairing is heterogenetic, segregation would result from both bivalents and multivalents. In the trispecies hybrids *G.hirsutum-G.raimondii-G.sturtianum* ($A_hC_1D_hD_5$), and *G.thurberi-G.sturtianum-G.hirsutum* ($A_hC_1D_hD_1$) where there is competition for pairing, the D_h chromosomes of *G. hirsutum* are expected to pair mostly with

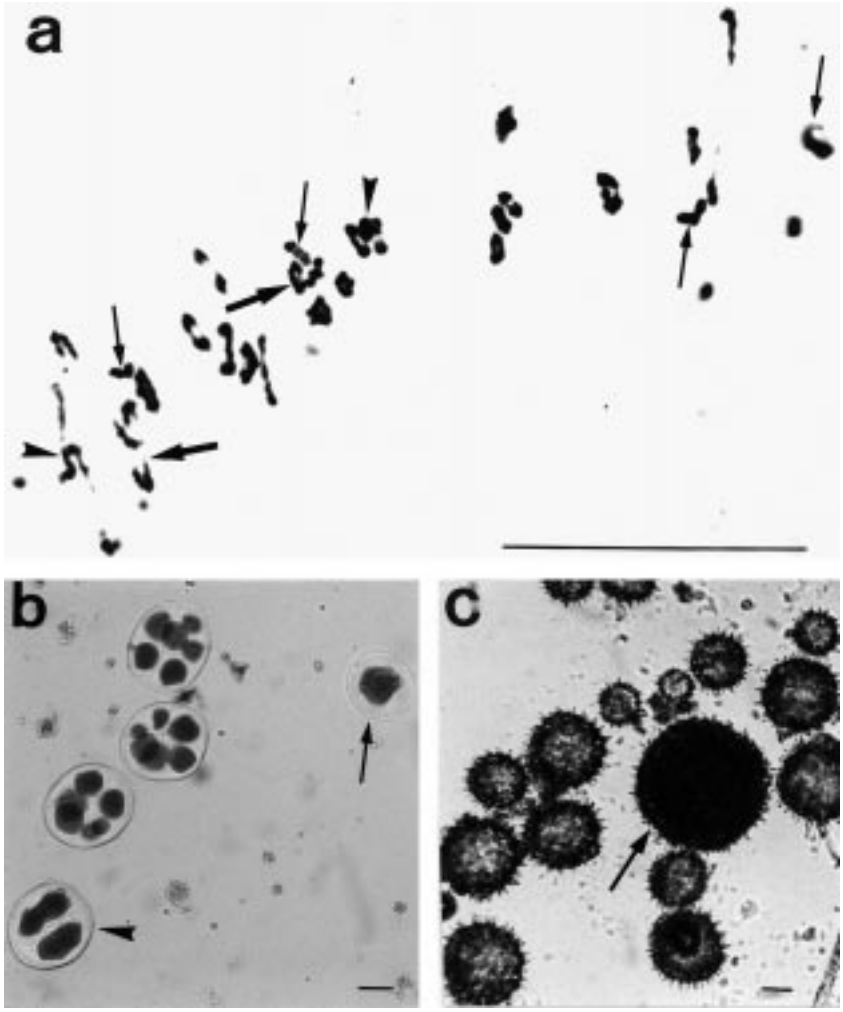

Figure 3a–c. Meiotic observations in the trispecies hybrids and the backcross progenies. (a) Metaphase-I cell of a BC1 plant TSH × C2 (S1) showing 7I + 16II + 2III + 2IV. Trivalents are marked with thick arrows, quadrivalents with arrowheads and large univalents (chromosomes C) with thin arrows. (b) Monad (arrow), diad (arrowhead) and tetrads with micronuclei in trispecies hybrid TSH. (c) pollen grain and micro-pollen in TSH; only large red grains (arrow) were considered fertile. Scale bar = 10 μm.

their homeologous D_1 and D_5 chromosomes of the bridge species. Then, A_h chromosomes of *G. hirsutum* will be mostly confronted with C_1 chromosomes of *G. sturtianum*. Multivalent associations are also expected due to segmental interchanges that took place between A, D and C genomes during *Gossypium* evolution (Maréchal, 1974). The observed rod bivalents and heteromorphic multivalents suggests intergenomic exchanges. Ring bivalents are probably issued from autosyndesis and/or pairing between homeologues.

In cotton breeding programs attempting the introgression of the glandless-seed and glanded-plant trait, only Shuijin and Biling (1993) developed a trispecies hybrid *G. arboreum* × *G. bickii* × *G. hirsutum*. These authors reported a chromosome configuration of 2n = 4x = 52 = 41.01I + 4.54II + 0.57III for the trispecies hybrid. The mean numbers of bi- and multivalent associations in TSH and HRS are significantly higher than what was observed by Shuijin and Biling (1993). Because there is no previous data on BC1, BC2 and BC3 progenies derived from cotton trispecies hybrids including an Australian wild species, there are no comparable pairing data to evaluate our results in the backcross generations. Nevertheless, in some meiotic plates of BC3 plants (e.g. BC2/1 × StamF/4 and BC2/1 × stamF/12), all the chromosomes were involved in pairing. This high level of chromosome

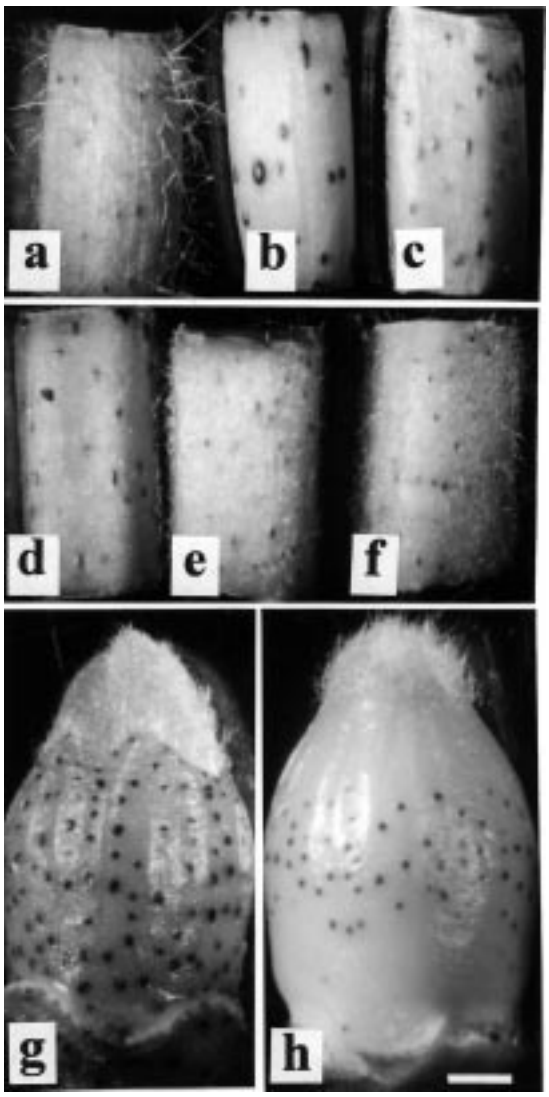

Figure 2a–h. Gossypol glanding patterns in organs of *G. hirsutum*, *G. sturtianum* and hybrids. (a) *G. hirsutum* StamF. (b) *G. sturtianum*. (c) Trispecies hybrid *G. thurberi–G. sturtianum–G. hirsutum* (TSH). (d, e) BC1 plants derived from pollination of TSH. (f) BC2 plant derived from TSH. (g, h) Flower buds of two BC3 hybrids derived from the same cross (BC2/1 × StamF). Scale bar = 1.25 mm.

associations should allow exchanges and recombinations.

By considering that nearly all chromosomes arms were chiasmate in *G. hirsutum*, Menzel et al. (1985) predicted an average of 60 chiasmata per PMC. But Srivastava (1980) directly scored a mean frequency of chiasmata of 21.20 per PMC in *G. hirsutum*. The mean number of chiasmata scored in this study ranged from 33.25 to 48.67 indicating that a relatively high level of recombination may occur in such hybrids. Moreover, the introgression of the wild parents specific markers was evidenced at the molecular level by Random amplified polymorphic DNA (Mergeai et al., 1998). The inheritance of most of these markers fit the expected mendelian segregation ratio, showing that molecular markers like RAPD should help monitoring parental genes introgression in the backcross generations.

Cotton breeders have been searching for methods to develop plants with high gossypol content. The main problem encounterred was the concomittant increase of gossypol level in seed of high gossypol plants, limiting cottonseed utilization (Pauly, 1979; Calhoun, 1997). In this study, most of the seeds produced had a lower level of gossypol glands than in cultivated cottonseed, while all the vegetative parts of the plants were glanded. A great variability for glands density was revealed between organs and also between plants. Cotton breeders can exploit this variability to develop an improved cultivar of cotton having the desired trait. Furthermore, correlation of seed gland level with leaf, stem, bract, and calyx gland density was low, while significant association were found between gland density of leaf, bract and calyx.

As far as we know, the genetic control of the glandless-seed and glanded-plant trait in *G. sturtianum* remains unknown. Trials are in progress to characterize the chromatin of *G. sturtianum* transmitted to the hybrid plants. In view of this, we are currently using RFLP probes specific to cotton chromosomes (Reinisch et al., 1994). Beside the main objective of this study, some other characters of the wild parents might be of value if transferred in a commercial cotton. These characters include high fiber strength, drought, frost, cold and disease resistance (Brown & Menzel, 1950; Louant, 1971; Rooney et al., 1991). The perennial nature of the hybrid plants obtained makes them particularly interesting for cotton breeding programs.

Acknowledgements

We are very grateful to Eugene Journée for care of plants in the greenhouse and to Dr Alain Maquet for critical reading of the manuscript. This research was supported by the convention 2.4565.95 of the Belgian ‘Fonds de la recherche fondamentale collective’. The PhD scholarship of the first author was provided by the Ministry of Scientific Research of Ivory Coast.

References

Alford, B.B., G.U. Liepa & A.N. Vanbeber, 1996. Cottonseed protein: What does the future hold? Plant Foods for Hum Nutr 49: 1–11.

Altman, D.W., R.D. Stipanovic & A.A. Bell, 1990. Terpenoids in foliar pigment glands of A, D, and AD genome cottons: Introgression potential for pest resistance. J Hered 81: 447–454.

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

Bell, A.A., R.D. Stipanovic, D.H. O'Brien & P.A. Fryxell, 1978. Sesquiterpenoid aldehyde quinones and derivatives in pigment glands of *Gossypium*. Phytochemistry 17: 1297–1305.

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

Brubaker, C.L., C.G. Benson, C. Miller & 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.

Calhoun, D.S., 1997. Inheritance of high glanding, an insect resistance trait in cotton. Crop Sci 37: 1181–1186.

Fisher, G.S., A.W. Frank & J.P. Cherry, 1988. Total gossypol content of glandless cottonseed. J Agric Food Chem 36: 42–44.

Forman, L., S. Mathews & C.C. King, 1991. New food, feed uses for glandless cottonseed. Inform 2: 737–739.

Fryxell, P.A., L.A. Craven & J.McD. Stewart, 1992. A revision of *Gossypium* sect. Grandicalyx (Malvaceae), including the description of six new species. Syst Bot 17: 91–114.

Garas, N.A. & A.C. Waiss Jr., 1986. Differential accumulation and distribution of antifungal sesquiterpenoids in cotton stems inoculated with *Verticillium dahliae*. Phytopathology 76: 1011–1017.

Gill, M.S. & Y.P.S. Bajaj, 1984. Interspecific hybridization in the genus *Gossypium* through embryo culture. Euphytica 33: 305–311.

Hedin, P.A., W.L. Parott & J.N. Jenkins, 1992. Relationships of glands, cotton square terpenoid aldehydes, and other allochemicals to larval growth of *Heliothis virescens* (Lepidoptera: Noctuidae). J Econ Entomol 85: 359–364.

Hron, R.J., P.J. Wan & M.S. Kuk, 1996. Ethanol vapor deactivation of gossypol in cottonseed meal., J Am Oil Chem 73: 1337–1339.

Kadan, R.S., D.W. Freeman, G.M. Ziegler & J.L. Spadaro, 1979. Air classification of defatted, glanded cottonseed flours to produce edible protein product. J Food Sci 44: 1522–1524.

Khoshkhoo, N., P.A. Hedin & J.C. McCarty, 1994. Terpenoid aldehydes in root-knot nematode susceptible and resistant cotton plants. J Agric Food Chem 42: 204–208.

Liadakis, G.N., A. Floridis, C. Tzia & V. Oreopoulou, 1993. Proteins isolates with reduced gossypol content from screw-pressed cottonseed meal., J Agric Food Chem 41: 918–922.

Louant, B.P., 1971. Recherche sur les possibilités d'améliorer le cotonnier par l'introgressin directe de caractères provenant de *Gossypium raimondii* Ulbr. PhD thesis. Univ. Sci. Agron. Gembloux Belgium.

Lusas, E.W. & G.M. Jividen, 1987. Glandless cottonseed: A review of the first 25 years of processing and utilization research. J Am Oil Chem 64: 839–854.

Maréchal, R., 1974. Analyses de la conjugaison méiotique chez les hybrides triploïdes entre *Gossypium hirsutum* L. et les espèces sauvages Australiennes. Bull. Rech. Agron. Gembloux 9: 193–204.

Marquié, C., 1987. Food use of glandless cotton derivatives. Coton Fibres Trop 42: 65–73.

McMichael, S.C., 1954. Glandless boll in upland cotton and its use in the study of natural crossing. Agron J 46: 527–528.

Menzel, M.Y., K.L. Richmond & B.J. Dougherty, 1985. A chromosome translocation breakpoint map of *Gossypium hirsutum* genome. J Hered 76: 406–414.

Mergeai, G., I. Vroh Bi, J.P. Baudoin & P. du Jardin, 1998. Use of Random Amplified Polymorphic DNA (RAPD) markers to assist wide hybridizaton in cotton. In: Y.P.S. Bajaj (Ed), Biotechnol. in Agric. and Forest. Vol. 42, pp 121–139. Springer Verlag Berlin Heidelberg (in press).

Mohan, P., P. Singh, S.S. Narayanan & R. Ratan, 1994. Relation of gossypol-gland density with bollworm incidence and yield in tree cotton (*Gossypium arboreum*). Indian J Agric Sci 64: 691–696.

Parrott, W.L., J.N. Jenkins, J.E. Mulrooney, J.C. McCarty & R.L. Shepherd, 1989. Relationship between gossypol gland density on cotton squares and resistance to tobacco budworm (Lepidoptera: Noctuidae) larvae. J Econ Entomol 82: 589–592.

Pauly, G., 1979. Les glandes à pigment du cotonnier: aspect génétique et sélection des variétés glandless et high gossypol Coton Fibres Trop 34: 279–402.

Rahma, E.H. & R.M.S. Narasinga, 1984. Gossypol removal and functional properties of protein produced by extraction of glanded cottonseed with different solvents. J Food Sci 49: 1057–1060.

Rajarajeswari, V. & V. Subbarao, 1997. Gossypol glands in relation to resistance to bollworm (*Helicoverpa armigera*) in upland cotton (*Gossypium hirsutum*). Indian J Agric Sci 67: 293–295.

Reinisch, A.J., J. Dong, C.L. Brubaker, D.M. Stelly, J.F. Wendel & A.H. Patterson, 1994. A detailed RFLP map of cotton, *Gossypium hirsutum* × *Gossypium barbadense*: Chromosome organization and evolution in a disomic polyploid genome. Genetics 138: 829–847.

Rooney, W.L., D.M. Stelly & D.W. Altman, 1991. Identification of four *Gossypium sturtianum* monosomic alien addition derivatives from a backcrossing programm with *G. hirsutum*. Crop Sci 31: 337–341.

Shuijin, Z. & L. Biling, 1993. Studies of introgression of the glandless seeds-glanded plant trait from *Gossypium bickii* into cultivated upland cotton (*G. hirsutum*). Coton Fibres Trop 48: 195–199.

Srivastava, H.R., 1980. Correlation between chiasma frequency and quantitative traits in upland cotton (*Gossypium hirsutum* L.). Theor Appl Genet 56: 113–117.

Stewart, J.McD. & C.L. Hsu, 1977. In-ovulo embryo culture and seedling development of cotton (*Gossypium hirsutum* L.). Planta 137: 113–117.

Stipanovic, R.D., A.A. Bell, M.E. Mace & C.R. Howel, 1975. Antimicrobial terpenoids of *Gossypium*: 6-metoxygossypol and 6,6'-dimetoxygossypol. Phytochemistry 14: 1077–1081.

Vix, H.L.E., P.H. Eaves, H.K. Gardner & M.G. Lambou, 1971. Degossypolized cottonseed flour. The liquid cyclone process. J Am Oil Chem Soc 48: 611–615.

Vroh Bi, I., P. du Jardin, G. Mergeai & J.P. Baudoin, 1997. Optimization and application of RAPD (random amplified polymorphic DNA) in a recurrent selection programme of cotton (*Gossypium* spp.). Biotechnol Agron Soc Environ 1: 142–150.

Zhuge, Q., E.S. Posner & C.W. Deyoe, 1988. Production study of a low-gossypol protein product from cottonseed. J Agric Food Chem 36: 153–155.