Production of High Gossypol Cotton Plants with Low Gossypol Seed from Trispecies Hybrids Including *G. sturtianum* Willis

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ABSTRACT

Two tri-species hybrids that include G. hirsutum L. (2n=4X=52 (AD genome)) were created to develop upland cotton plants with low gossypol seed and high gossypol aerial parts. G. sturtianum Wil. was used as the donor parent (2n=2x=26, genome C1) and a wild American diploid cotton, G. thurberi Tor. (2n=2x=26, genome D1) or G. raimondii Ulb. (2n=2x=26 genome D5) as bridge species. Both tri-species hybrids were backcrossed to different G. hirsutum varieties, originating from Central and West Africa to produce BC_1, BC_2, selfed BC_2 and BC_3 seeds. Growth regulators applications at flowering, in vitro culture of the mature seed embryos and grafting of the more perturbed hybrids on vigorous G. hirsutum seedlings were necessary to obtain a large number of viable hybrid material. A drastic reduction of the gossypol gland density was expressed by at least 25% of the seeds of each backcross generation while the aerial parts of the resulting plants were normally glanded. Mortality rates of germinating seed and young plantlets were very high (76%) for BC_1 material but decreased markedly in following generations. Cytogenetic observations confirmed the soundness of the introgression strategy. Both tri-species hybrids and several BC_1 and BC_2 genotypes issued from nearly totally glandless seeds were fertile and showed high frequencies of multivalent and chiasma formations at metaphase I, indicating important genetic material exchanges. These plants constitute very interesting genetic stocks to develop commercial glanded cotton cultivars with low gossypol seed.

Introduction

Wild Australian diploid cottons belonging to *Sturtia* and *Hibiscoidea* sections of the genus *Gossypium* produce gossypol free seeds while gossypol glands are present in the rest of their aerial parts (Fryxell, 1965; Brubaker *et al.*, 1996). The introgression into upland cotton of the mechanism limiting the production of gossypol in the seeds is highly desirable because it would facilitate the exploitation of cotton as a food crop whilst preserving one of its natural defense mechanism against insect pests. Gossypol and other terpenoid aldehydes found in all tissues of cultivated cotton are toxic to most of the animal species including insects and, unfortunately, humans (Altman *et al.,* 1990; Jaroszewski, 1998). Three different crossing schemes can be followed to obtain the introgression of the "glanded plant - glandless seed" trait of Australian species to *G. hirsutum* (Mergeai, 1994). Two of them give rise to tri-specific hybrids and the last one allows the direct exploitation of bi-specific hybrids. As the direct crossing strategies between *G. hirsutum* and Australian diploids was not very successful (Dilday, 1986; Altman *et al.,* 1987; Rooney *et al.,* 1991), the tri-specific method was used. In these hybrids, obtained by bridge crosses, all the chromosomes of the Australian donor species are confronted with the genome of *G. hirsutum* with a high probability of recombination. Given the closer phyletic relationships existing between Asiatic (A genome) and Australian species (C genome) (Endrizzi *et al.,* 1985) the wild American diploids were used as a bridge to create ACDD tri-specific hybrids.

Material and methods

Two three-species hybrids including *G. hirsutum* L. (2n = 4 x = 52, (AD)1 genome) were created using *G. sturtianum* Willis as donor parent (2n = 2x = 26, genome C1) and a wild American diploid cotton (*G. thurberi* Tor. (2n = 2x = 26, D1 genome) or *G.*

raimondii Ulb. (2n = 2x = 26, D5 genome) as bridge species. These hybrids are respectively designated by TSH for [(*G.thurberi* x *G. sturtianum*) doubled x *G. hirsutum*, AhC1DhD1] and HRS for [(*G. hirsutum* x *G. raimondii*) doubled x *G. sturtianum*, AhC1DhD5]. The intermediate bispecific hybrids were doubled by colchiploidization as described in Beasley (1940) and the schemes to create tri-specific hybrids are detailed in Mergeai *et al.* (1998).

Both trispecific hybrids were backcrossed with different *G. hirsutum* glanded varieties originating from Central (NC8, C2) and West Africa (Stamf) with or without application of a growth regulator solution (50 mg/l NOA – 100 mg/l GA) on the ovary just after pollination to produce BC_1, BC_2, BC_2S_1 (selfed BC_2) and BC_3 seeds.

The gland density was assessed after removing seed integument on soaked kernels and according to a visual scale ranging from 0 for totally glandless to 10 for totally glanded seeds (Mergeai *et al.*, 1997). After this observation, the seeds were either planted directly in jiffy-pots containing a mixture of sand, peat and compost in equal proportions, or transferred to the *in vitro* culture media developed by Stewart and Hsu (1977) for cotton germinated embryos. When necessary, the plantlets were grafted on vigorous *G. hirsutum* seedlings. Adult plants were cultivated in greenhouses at Gembloux.

Pollen mother cells (PMC's) at metaphase I (M1) and pollen grains were prepared by the standard squash technique in 1.5 % acetocarmine (Vroh *et al.*, 1998a). For each PMC, the chromosome configurations and the number of chiasmata were recorded. A total of 56 PMC's were examined for each hybrid. Pollen stainability was assessed on 1,000 pollen grains per genotype.

Results and discussions

A rather similar expression of the "glanded-plant and glandless-seed" trait was observed in the bi-specific (*G.thurberi* x *G. sturtianum*) and trispecific (TSH and HRS) allotetraploids containing *G. sturtianum*. The seed gland density of these combinations was reduced by more than 90 % compared to their American diploid and tetraploid parents and the glands were mainly concentrated on the edge of the cotyledons. After germination, the number of glands increased to reach a normal density on the aerial parts of the plants. The tri-specific hybrid seeds produced by Shuijing and Biling (1993) from the backcross of the bi-specific allotetraploid *G. arboreum* L. x *G. bickii* Prokh. ($A_2A_2G_1G_1$) with *G. hirsutum* showed a similar reduction in gossypol gland density. A better expression of the repressive mechanism was observed by Mergeai (1992) in the *G. sturtianum* x *G. arboreum* synthetic bi-specific allotetraploid ($A_2A_2C_1C_1$) whose seeds where almost totally devoid of gossypol glands. As the major loci (GL_2, GL_3) that control gossypol synthesis in cotton are found respectively in A_h and D_h subgenomes (Lee, 1965), we can assume that the repressive mechanism of Australian cotton species is less effective as regards genome D (locus GL_3) than genome A (locus GL_2). As expected, both trispecific hybrids had 52 chromosomes. The mean numbers of bi- and multivalent associations in TSH (15.34 II + 0.93 III + 0.69 IV + 0.26 VI) and HRS (17.03 II + 0.82 III + 0.15 IV + 0,07 VI) were significantly higher than what was observed by Shujing and Biling (1993) in [(*G. arboreum* x *G. bickii*) doubled x *G. hirsutum*, $A_2G_1A_hD_h$] trispecific hybrid (4.54 II + 0.57 III + 0.41 IV). The rather high frequencies of pairing observed in TSH and HRS confirm the soundness of the introgression strategy followed. They indicate important genetic material exchanges between *G. hirsutum* chromosomes and those of the wild diploid

species constituting the tri-specific hybrids. The pairing frequencies observed in our tri-specific hybrids are similar to the data obtained by Louant and Maréchal (1975) in tri-specific hybrids involving *G. hirsutum* (A_hD_h) with a B and a D diploid and are not much lower than what was observed by Brown and Menzel (1950) in hybrids combining an American (genome D) and an Asiatic (genome A) species to *G. hirsutum*. Provided an American diploid is used as bridge species, this type of interspecific structure can thus be productive to improve upland cotton even with C genome species that are phylletically more distant of *G.hirsutum* genome than A, D or B diploids. Numbers of individuals produced from BC hybrid seeds are given in Table 1. The backcross of TSH and HRS hybrids with *G. hirsutum* glanded varieties started in 1991. At the beginning no growth regulators were applied on the flowers to prevent boll shedding. In these conditions, the backcross success rate was nil for TSH and extremely low for HRS (1 seed for more than 100 crosses). In order to improve this rate, the growth regulator formula proposed by Altman (1988) on pollinated flowers was applied. This resulted in a significant number of viable backcrossed seeds being obtained each year from the two tri-specific hybrids (41 from 1991 to 1993, 17 in 1994, 26 in 1995 and 30 in 1996). On an average, about 15 crosses were necessary to obtain one seed with both hybrids. All the gland patterns, ranging from "0" (totally glandless) to "10" (similar to *G .hirsutum* gland density) were observed in the BC_1 seeds. Data on seed gland patterns distribution in the tri-specific hybrid derivatives are given in Table 2. Among the 114 BC_1 seeds, nine (8 %) were totally glandless, three from HRS and six from TSH. The frequency distribution of the gland density in the rest of the BC_1 seeds was asymetric : low and intermediate gland patterns, ranging from 2 to 5, were the most frequent.

A very important degree of variability was observed in the number of adult plants obtained from the BC_1 seeds produced by TSH and HRS according to their year of cultivation (Table 1). In 1994, all the BC_1 seeds produced the previous years by the HRS tri-specific hybrid were planted in jiffy pots containing an unsterilised substrate. Among the 17 seeds sown, only 6 gave rise to adult plants. The rest, including the two totally glandless seeds produced by HRS hybrid, did not germinate or died at a very early stage. Of six adult plants eventually produced, two died subsequently. Considering the very low survival rate obtained for the first HRS BC_1 seeds, the rest of our BC_1 seeds were cultivated *in vitro* on the rooting medium developed by Stewart and Hsu (1977). This allowed the production of 15 adult plants from the 24 BC_1 TSH seeds cultivated in 1994. The 43 BC_1 mature embryos of both trispecific hybrids cultivated *in vitro* during the next two years did not germinate and decayed by rotting in the culture substrate. This failure in germination was attributed to the dormancy of the seeds that were cultivated less than 6 months after harvest. To remove this supposed dormancy cultivation of the seeds in 1997 was delayed by 4 months (cultivation in early July instead of early March) and all the seeds were soaked for 2 minutes in a 70°C water bath before *in vitro* cultivation (Stewart, 1997, pers comm). These measures resulted in a very important improvement (from 0 to more than 50 %) survival rate of the BC_1 seeds. Only one of the 4 BC_1 glandless seeds produced by TSH in 1993 gave rise to a viable plant presenting a normal gland density in its aerial parts. Due to its very poor growth and development, it was necessary to graft this plant (TSH-BC_1/93/5) on a vigorous *G. hirsutum* seedling. The rest

of the glandless embryos degenerated after a few days of *in vitro* culture.

All the first BC_1 plants issued from low level glanded seeds (glanding classes "0" to "3") were systematically backcrossed with glanded varieties of *G. hirsutum*. Among them, only two (TSH-BC_1/93/5 and HRS-BC_1/92/1) produced BC_2 seeds. Most of these seeds showed a low or intermediate gossypol gland density (Table 2). Only 6 adult plants were produced from a total of 21 BC_2 seeds. Two of these plants gave 18 BC_2S_1 and 19 BC_3 seeds, most of them with low and intermediate gland levels. The fertility of the backcross derivatives improved markedly with advanced generations. Pollen stainability was very low in tri-specific hybrids and BC_1 derivatives (less than 10 %) but increased to about 60 % in fertile BC_2 plants and up to 100 % in best BC_3 materials. On an average, four crosses were necessary to obtain one BC_2 seed while one backcross of a fertile BC_2 plant gave about 5 BC_3 seeds. BC_2S_1 plants were less fertile than BC_3 materials (about two crosses were necessary to get one seed). Some of the BC_3 plants issued from low gossypol seeds are self fertile even without application of growth regulators at pollination. Their progeny will be used to assess the determinism of the "low-gossypol seed and high-gossypol plant" character.

Cytogenetic analysis of the trispecific hybrid derivatives showed important variability in chromosome number among BC_1 and BC_2 plants. On a total of 8 BC_1 plants analyzed, 3 had 2 to 4 supernumerary chromosomes and one had 3 chromosomes missing. The rest were euploid with 2n=4x=52 chromosomes (Vroh Bi *et al.*, 1998a, 1998b). The two BC_1 plants that produced BC_2 derivatives were characterized by a high level of chromosome associations (20.61 II + 0.69 III + 0.77 IV for TSHxNC8/93/5 and 22.56 II + 0.30 III + 0.10 IV for HRSxC2/92/1). They showed also a high mean number of chiasmata, suggesting a high frequency of genetic material exchange (Table 3).

Two of the three BC_2 plants analyzed cytogenetically (HRS-BC_2/95/1 and HRS-BC_2/95/3) were euploid. The last one (HRS-BC_2/95/2) had only 49 chromosomes and was totally sterile. Cytogenetic analysis of BC_2S_1 and BC_3 materials are in progress. Preliminary data show that most of these genotypes are euploid (2n = 4x = 52) and some are aneuploid with 2n = 4x + 2 = 54.

Conclusions

If an American diploid cotton is used as bridge species, the development of tri-specific hybrids is a promising way to improve upland cotton using a C genome diploid donor parent. In this hybrid, pairing affinity between A and C genomes is sufficient to allow exchanges of genetic material and the production of introgressed euploid plants. A drastic reduction of the gossypol gland density was expressed by at least 25 % of the seeds of each backcross generation of TSH and HRS hybrids while the aerial parts of the resulting plants were normally glanded. Both tri-specific hybrids and several BC_1 and BC_2 genotypes issued from nearly totally glandless seeds were fertile and showed high frequencies of multivalent and chiasma formations at metaphase I, indicating important genetic material exchanges. These plants constitute very interesting genetic stocks to develop commercial glanded cotton varieties with low gossypol seeds.

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Table 1. Number of individuals produced from BC_1, BC_2, BC_2S_1 and BC_3 hybrid seeds.

Year	No Cultivated seeds	No adult Plants produced	No Cultivated seeds	No adult plants produced
		BC_1		
	HRS		TSH	
1994	17	6	24	15
1995	13	0	4	0
1996	9	0	17	0
1997	12	10	18	8
Total	51	16	63	23
		BC_2		
	HRS-BC_1/92/1		TSH-BC_1/93/5	
1994				
1995	6	0	-	-
1996	3	3	2	0
1997	8	1	2	2
Total	17	4	2	2
		BC_3		
	HRS-BC_2/95/1		HRS-BC_2/95/3	
1994				
1995				
1996				
1997	13	10	6	5
Total	13	10	6	5
		BC_2S_1		
	HRS-BC_2/95/1		HRS-BC_2/95/3	
1997	15	10	3	1
Total	15	10	3	1

Table 2. Gossypol gland density distribution in trispecific hybrids backcross derivatives.

Glanding Classes	BC_1		BC_2		BC_3		BC_2S_1	
	HRS	TSH	HRS-BC_1 /92/1	TSH-BC_1 /93/5	HRS-BC_2 /95/1	HRS-BC_2 /95/3	HRS-BC_2 /95/1	HRS-BC_2 /95/3
0 – 3	23	25	6	2	4	-	8	2
4 – 6	19	23	10	2	6	5	5	-
7-10	9	15	1	-	3	1	2	1
Total	51	63	17	4	13	6	15	3

Table 3. Mean frequencies and ranges of chromosome associations and chiasmata observed in the trispecific hybrids and their fertile derivatives.

Genotypes	Chromosome Number	I	II	III	IV	V	VI	Chiasmata
TSH	52	15.07 (10-24)*	15.34 (11-20)	0.82 (0-3)	0.46 (0-2)	-	0.26 (0-2)	37.30 (32-45)
HRS	52	14.42 (7-21)	17.03 (12-21)	0.93 (0-3)	0.15 (0-1)	-	0.07 (0-1)	36.88 (31-46)
TSH-BC_1/93/5	52	5.26 (1-11)	20.61 (12-24)	0.79 (0-3)	0.77 (0-3)	-	-	50.38 (44-59)
HRS-BC_1/92/1	53	6.45 (3-11)	22.56 (19-25)	0.30 (0-3)	0.10 (0-1)	-	-	42.76 (35-51)
HRS-BC_2/95/1	52	3.83 (1-9)	23.61 (20-25)	0.31 (0-1)	-	-	-	42.12 (34-50)
HRS-BC_2/95/3	52	5.52 (2-8)	23.13 (21-25)	0.07 (0-1)	-	-	-	38.96 (35-46)

*The range of each configuration is given in brackets