Exploitation of trispecific hybrids to introgress the glandless seed and gloved plant trait of *Gossypium sturtianum* Willis into *G. hirsutum* L.

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Two different trispecific hybrids were developed in order to introgress the “glandless seed-gloved plant” trait of *Gossypium sturtianum* Will. (2n = 2x = 26, C₁ genome) into the main cultivated cotton species (*Gossypium hirsutum* L., 2n = 4x = 52, (AD)₁ genome) using either *Gossypium raimondii* Ulb. (2n = 2x = 26, D₃ genome) or *Gossypium thurberi* Tor. (2n = 2x = 26, D₁ genome) as bridge species. Both trispecific hybrids were backcrossed with two *G. hirsutum* varieties (C₂ and NC₈) originating from Zaire. Observation of the trispecific seeds pointed out the incomplete expression of the seed gossypol glands repressive mechanism of *G. sturtianum* when its chromosomes set is confronted with the D genome. The glandless trait was expressed in a rather high proportion of the BC₁ seeds: 6 out of 41. Only one of the six BC₁ glandless seeds gave rise to a viable gloved plant. Cytogenetic observations of both trispecific hybrids and of the introgressed plant confirmed the soundness of the introgression strategy followed. All these plants were euploid (2n = 4x = 52) and showed high frequencies of multivalent and chiasma formations at metaphase I indicating important genetic material exchanges. All the plants issued from nearly totally glandless seeds will be used in a backcrossing program with *G. hirsutum* to produce commercial varieties of upland cotton expressing the “glandless seed-gloved plant” trait.

**Keywords.** Cotton, *Gossypium*, interspecific hybridization, gossypol glands.

**INTRODUCTION**

The presence of lysigenous glands filled with gossypol and other terpenoid aldehydes in most tissues of cultivated cotton induces natural resistance to insect pests (Altman *et al.*, 1990). However, the release of terpenoid aldehydes during the crushing of cotton seed kernels renders oil and protein meals toxic to non-ruminant animals, including humans. In the Australian wild cotton species belonging to *Sturtia* and *Hibiscoides* sections of the genus *Gossypium*, the formation of gossypol glands is controlled by a repressive mechanism which acts until the cotyledons open and the plantlet begin to produce chlorophyll (Fryxell, 1965; Brubacker *et al.*, 1996). The seeds of these Australian cottons are thus totally gossypol free while their aerial parts contain protective pigment glands. The main objective of our work is to introgress
this trait from the Australian wild diploid species *Gossypium sturtianum* Will. (C₁ genome) into the main cultivated tetraploid cotton (*Gossypium hirsutum* L., (AD₁) genome).

**MATERIAL AND METHODS**

The paraphyletic and aphyletic introgression methods (Mergeai, 1994; Mergeai *et al.*, 1995) were followed to create two trispecific hybrids including respectively *G. hirsutum* 2(A₅D₅) as recipient species, *G. sturtianum* (2C₁) as donor parent and two American wild diploids *Gossypium thurberi* Tod. (2D₁) and *Gossypium raimondii* Ulbr. (2D₅) as bridge species. Both TSH (*thurberi × sturtianum × hirsutum*) and HRS (*hirsutum × raimondii × sturtianum*) hybrids were backcrossed to different *Gossypium hirsutum* varieties originating from Zaire (C₂ and NC₈). Figures 1 and 2 show the crossing schemes followed to obtain the BC₁ genotypes. It was necessary to treat the flowers with growth regulators (50 mg/l naphthoxyacetoc acid–100 mg/l gibberelic acid) to produce BC₁ seeds from the trispecific hybrids. Given the very low germination vigour shown by the backcross materials, most of the mature embryos were cultivated in *vitro* on the medium of Stewart and Hsu (1977) after removing the seed coat and assessment of their gland density. When necessary, the plantlets were grafted on vigorous *G. hirsutum* seedlings. The gland density of the hybrid embryos was evaluated under a stereo microscope Wild M3 Z according to a score scale ranging from 0 for completely glandless to 10 for fully glanded. After being fixed for 72 hours in Carnoy's solution (95% ethanol:chloroform:glacial acetic acid, 6:3:1 v:v:v) flower buds were used to perform meiotic studies. Microsporocyte squashes were stained with 1.5% acetocarmine solution and examined with a Nf Jena (Carl Zeiss) microscope.

**RESULTS AND DISCUSSION**

In all the hybrid structures containing *G. sturtianum* (i.e. in the *thurberi × sturtianum* allotetraploid and in the two trispecific hybrids TSH and HRS) we observed a rather similar expression of the “glanded plant–glandless seed” trait. The seeds presented a restricted number of glands that were mainly located on the edge of cotyledons (Figure 3). After germination, the number of glands increased to reach a normal density on the aerial parts of the plants. A similar observation was made by Shuijin and Biling (1993) on the seeds produced by crossing the bispecific allotetraploid *Gossypium arboreum × Gossypium bickii* with *G. hirsutum*. This means that the expression of the repressive mechanism seems to

![Diagram](image)

*Figure 1. Development and exploitation scheme of TSH (G. thurberi × G. sturtianum × G. hirsutum) trispecific hybrid — Production d'hybrides trispecifiques. Schéma de création de l'hybride TSH.*
be limited when the chromosome set of an Australian species is confronted with the D genome. Both trispecific hybrids were selfsterile and it was very difficult to produce seeds by backcrossing them with G. hirsutum. Without application of growth regulators, the backcross success rate was nil for TSH and extremely low for HRS (1 seed for more than 100 crosses). The application of the growth regulators mixture of Altman (1988) allowed the production of 41 backcrossed seeds (17 from HRS and 24 from TSH). On an average, about 17 crosses were necessary to obtain one seed with both hybrids. On the 41 seeds we observed, 6 were totally glandless, two from HRS and four from TSH. The frequency distribution of the gland density in the rest of the BC₁ seeds was characterized by an asymmetric bell curve (Figure 4). Intermediate glancing patterns, ranging from 4 to 7 were the most frequent.

After evaluation of their gland density, all the BC₁ seeds produced by the HRS trispecific hybrid were planted in jiffy pots containing a mixture of sand, peat and compost in equal proportions. Among the 17 seeds we sowed, only 6 gave rise to adult plants. The rest did not germinate or died at a very early stage. The two totally glandless seeds produced by the HRS hybrid did not germinate and decayed by rotting in the culture substrate. In order to improve the survival rate of interspecific material, we decided to cultivate in vitro all the remaining seeds. So after assessment of their gland density, the 24 seeds produced by the TSH hybrid were grown on the rooting medium developed by Stewart and Hsu (1977). Twenty of those seeds germinated and gave rise to plantlets that were sufficiently well developed to be transferred to normal growing conditions. Among these 20 plants, 15 survived and were transferred to greenhouses. Only one of the four totally glandless seeds we cultivated in vitro gave an adult plant presenting a normal gland density in its aerial parts. Among the three others, one did not develop at all and the rest began to grow slightly before degenerating after a few days of in vitro culture. The growth of the only surviving plant issued from a totally glandless seed (TSH × NC8/5) was very slow compared to most of the other materials. For this reason, we decided to graft it on a vigorous G. hirsutum seedling. This operation had a very positive effect on the development of the plant which began to flower abundantly a few months latter.

Due to the lack of flower buds in most of the BC₁ materials, we have only been able to perform cytogenetic analyses on the trispecific hybrids and on four of their BC₁ derivatives.

Both trispecific hybrids are euploid (2n = 4x = 52 chromosomes) and the mean numbers of bi- and multivalent associations in TSH (15.34 ± 0.49 II + 0.93 ± 0.17 III + 0.69 ± 0.14 IV + 0.26 ± 0.1 VI) and HRS (17.03 ± 0.49 II + 0.82 ± 0.19 III + 0.15 ± 0.07 IV + 0.07 ± 0.05 VI) are significantly higher than what was
Figure 3. Appearance of seeds observed under binocular microscope after removal of integuments and soaking in water for one hour (the line beside each seed represents 1 mm) — Aspect des graines observées au microscope binoculaire, après enlèvement des téguments et trempage à l’eau durant une heure (le trait au-dessus de chaque graine représente 1 mm).
CONCLUSION

We proved that the development of three species bridge crosses can be productive to improve upland cotton using a C genome diploid donor parent if an American diploid cotton is used as bridge species. In this structure, the remaining pairing affinity existing between A and C genomes is high enough to assure genetic material exchanges and the production of some introgressed euploid plants. Chromosome pairing frequencies are similar to the data observed with trispecific hybrids involving a B genome species (Louant, Maréchal, 1975). The application of growth regulators just after crossing, the systematic in vitro culture of the mature hybrids embryos and the grafting on vigorous G. hirsutum seedlings of the most perturbed hybrids are necessary to succeed this kind of program.

The expression of the seed gossypol glands repressive mechanism in an interspecific cotton hybrid involving A_h and D_h subgenome is a first step which permits to be rather optimistic about the possibility to transfer this trait into commercial upland cottons varieties. This genotype and all the plants issued from nearly totally glandless seeds will be involved in a backcrossing program with G. hirsutum in order to reach this goal.

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Bibliography


Exploitation of trispecific cotton hybrids including C genome species


