B A
S E

Biotechnol. Agron. Soc. Environ. 1997 **1** (4), 272–277

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

Guy Mergeai, Jean-Pierre Baudoin, Irié Vroh Bi

Unité de Phytotechnie des Régions intertropicales. Faculté universitaire des Sciences agronomiques de Gembloux. Passage des Déportés, 2. B-5030 Gembloux (Belgique). E-mail: mergeai@fsagx.ac.be

Received 6 May 1997, accepted 12 June 1997.

Two different trispecific hybrids were developed in order to introgress the "glandless seed-glanded plant" trait of *Gossypium sturtianum* Will. (2n = 2x = 26, C_1 genome) into the main cultivated cotton species (*Gossypium hirsutum* L., 2n = 4x = 52, $(AD)_1$ genome) using either *Gossypium raimondii* Ulb. (2n = 2x = 26, D_5 genome) or *Gossypium thurberi* Tor. (2n = 2x = 26, D_1 genome) as bridge species. Both trispecific hybrids were backcrossed with two *G. hirsutum* varieties (C_2 and NC_8) 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 BC_1 seeds: 6 out of 41. Only one of the six BC_1 glandless seeds gave rise to a viable glanded 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-glanded plant" trait.
Keywords. Cotton, *Gossypium*, interspecific hybridization, gossypol glands.

Exploitation d'hybrides trispécifiques en vue d'introgresser le caractère "graine sans glande – plante avec glandes" de *Gossypium sturtianum* Will. chez *Gossypium hirsutum* L. En vue d'introgresser le retard à la morphogenèse des glandes à gossypol de la graine de *Gossypium sturtianum* Will. (2n = 2x = 26, génome C_1) chez la principale espèce de cotonnier cultivé (*Gossypium hirsutum* L., 2n = 4x = 52, génome $(AD)_1$), deux hybrides trispécifiques ont été créés en utilisant respectivement comme espèce pont *Gossypium raimondii* Ulb. (2n = 2x = 26, génome D_5) ou *Gossypium thurberi* Tor. (2n = 2x = 26, génome D_1). Les deux hybrides trispécifiques ont été croisés avec deux variétés de *G. hirsutum* (C_2 et NC_8) originaires du Zaïre. L'observation des graines trispécifiques met en évidence l'expression incomplète du mécanisme répresseur de la formation des glandes à gossypol de *G. sturtianum* quand ses chromosomes sont confrontés au génome D. Plusieurs graines provenant du rétrocroisement des hybrides trispécifiques étaient totalement dépourvues de glandes à gossypol : 6 sur 41. Une seule d'entre elles a donné naissance à une plante adulte présentant une densité normale de glandes à gossypol. Les observations cytogénétiques réalisées sur les hybrides trispécifiques et leur descendance introgressée ont confirmé le bien-fondé de la stratégie d'introgression proposée. Toutes ces structures étaient euploïdes (2n = 4x = 52) et montraient des fréquences élevées de multivalents et de chiasmas en métaphase 1, indices d'importants échanges de matériel génétique. Toutes les plantes issues de graines totalement ou presque totalement démunies de glandes seront utilisées dans un programme de rétrocroisement avec *G. hirsutum* pour produire des variétés commerciales de cotonnier "upland" exprimant le retard à la morphogenèse des glandes à gossypol de la graine.
Mots-clés. Cotonnier, *Gossypium*, hybridation interspécifique, glandes à gossypol.

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 *Hibiscoidea* 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. (C_1 genome) into the main cultivated tetraploid cotton (*Gossypium hirsutum* L., $(AD)_1$ 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* $2(A_hD_h)$ as recipient species, *G. sturtianum* $(2C_1)$ as donor parent and two American wild diploids *Gossypium thurberi* Tod. $(2D_1)$ and *Gossypium raimondii* Ulbr. $(2D_5)$ as bridge species. Both TSH (*thurberi* × *sturtianum* × *hirsutum*) and HRS (*hirsutum* × *raimondii* × *sturtianum*) hybrids were backcrossed to different *Gossypium hirsutum* varieties originating from Zaire (C_2 and NC_8). **Figures 1** and **2** show the crossing schemes followed to obtain the BC_1 genotypes. It was necessary to treat the flowers with growth regulators (50 mg/l naphtoxyacetic acid–100 mg/l gibberellic acid) to produce BC_1 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

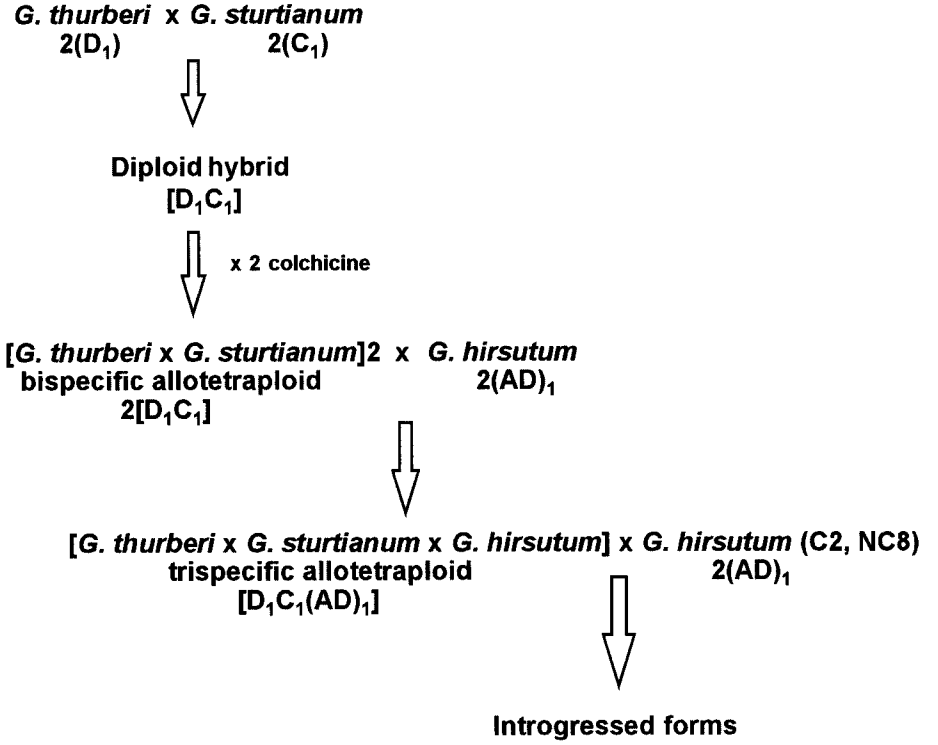


Figure 1. Development and exploitation scheme of TSH (*G. thurberi* × *G. sturtianum* × *G. hirsutum*) trispecific hybrid — *Production d'hybrides trispécifiques. Schéma de création de l'hybride TSH.*

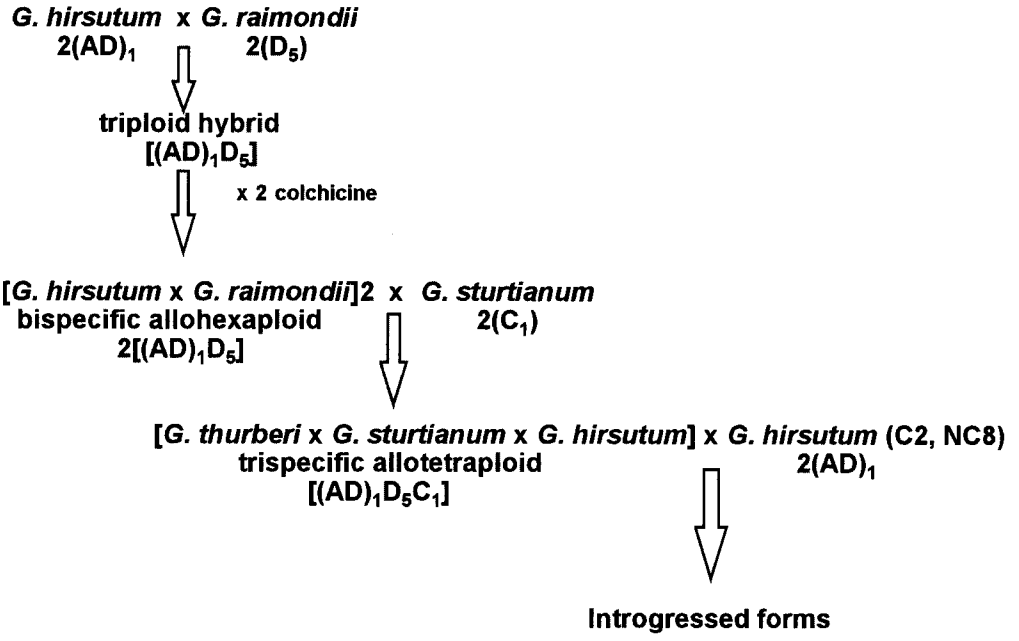


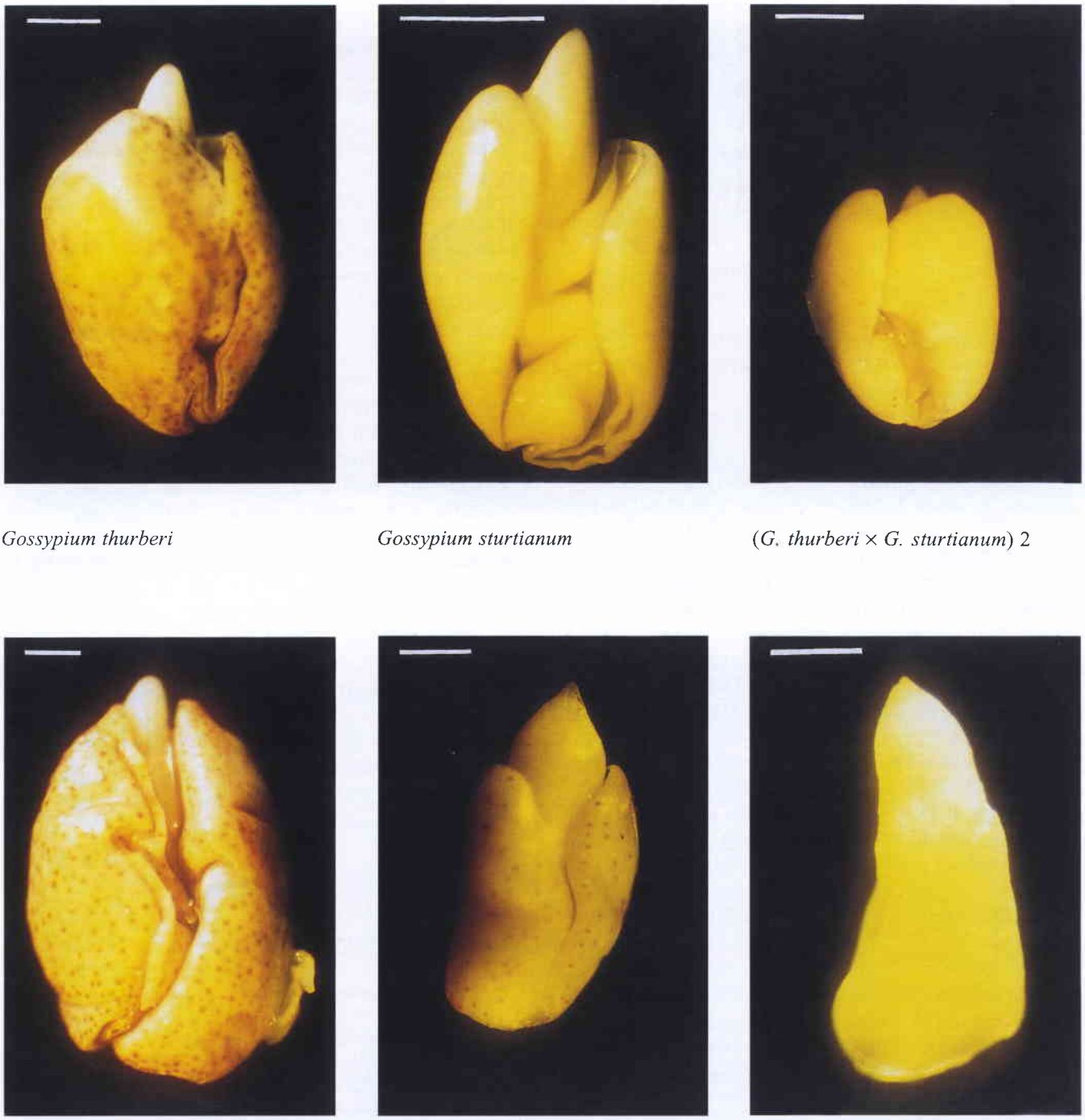
Figure 2. Development and exploitation scheme of HRS (*G. hirsutum* × *G. raimondii* × *G. sturtianum*) trispecific hydrid — *Production d'hybrides trispécifiques. Schéma de création de l'hybride HRS.*

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 BC_1 seeds was characterized by an asymmetric bell curve (**Figure 4**). Intermediate glanding patterns, ranging from 4 to 7 were the most frequent.

After evaluation of their gland density, all the BC_1 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 BC_1 materials, we have only been able to perform cytogenetic analyses on the trispecific hybrids and on four of their BC_1 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



Gossypium thurberi | *Gossypium sturtianum* | (*G. thurberi* × *G. sturtianum*) 2

Gossypium hirsutum (var. NC8) | TSH (*G. thurberi* × *G. sturtianum* × *G. hirsutum*) | TSH × NC8/5

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).*

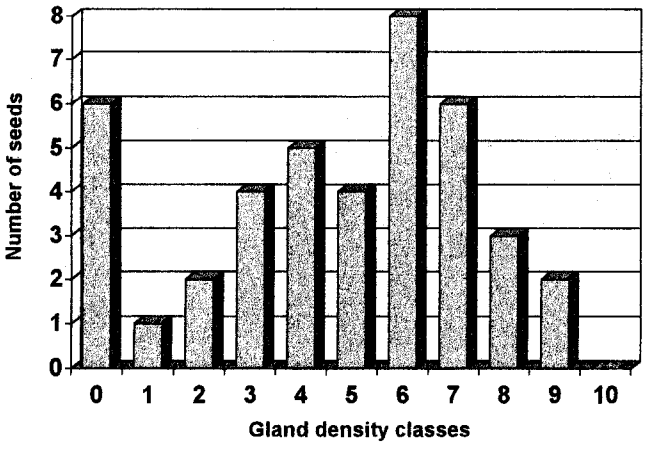


Figure 4. Gossypol gland density distribution in THS and HRS BC_1 seeds — *Distribution des densités de glandes à gossypol observée dans les graines BC_1 des hybrides TSH et HRS.*

observed by Shuijin and Biling (1993) in *arboreum* × *bickii* × *hirsutum* (ABH) trispecific hybrid (4.54 II + 0.57 III + 0.41 IV). The rather high pairing frequencies observed in TSH and HRS indicate genetic material exchanges between *G. hirsutum* chromosomes and those of the wild diploid species constituting the trispecific hybrids.

Two of the BC_1 plants were aneuploid: TSH × C2/10 with 2n = 4x + 2 = 54 and TSH × NC8/9 with 2n = 4x + 4 = 56. The BC_1 plants HRS × NC8/3 and TSH × NC8/5 were euploid (2n = 4x = 52). Among the BC_1 plants we analysed, TSH × NC8/5 is the only one issued from a totally glandless seed. To our knowledge, it is the first report of the expression of the glandless seed and glanded plant trait of *G. sturtianum* in a tetraploid cotton genotype including A_h and D_h subgenomes. The level of bi- and multivalent chromosome associations observed in TSH × NC8/5 was very high (20.61 ± 0.57 II + 0.69 ± 0.17 III + 0.77 ± 0.15 IV) compared to all the other interspecific genotypes. This genotype showed also the highest mean number of chiasmata (50.38 ± 0.63) among all plants under study, suggesting a high frequency of genetic material exchange. Based on the chromosome size, the univalent number of C_1 chromosomes of the donor parent in this BC_1 ranged from two to four but we were not able to identify all the chromosomes involved in conjugation. So, although the chiasma frequency was high, it is impossible to determine whether the expression of the glandless seed–glanded plant trait in the BC_1 is due to recombination or to the presence of C_1 univalents.

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.

Acknowledgment

This work was funded by the Belgian "Fonds de la Recherche Fondamentale Collective" through the convention 2.4565.95.

Bibliography

Altman DW (1988). Exogenous hormone applications at pollination for in vitro and in vivo production of cotton interspecific hybrids. *Plant Cell Rep.* **7**, 257-261.

Altman DW, Stipanovic RD, Bell AA (1990). Terpenoids in foliar pigment glands of A, D, and AD genome cottons: Introgression potential for pest resistance. *J. Hered.* **81**, 447-454.

Brubaker CL, Benson CG, Miller C, Leach DN (1996). Occurrence of terpenoid aldehydes and lysigenous cavities in the glandless seeds of Australian *Gossypium* species. *Aust. J. Bot.* **44**, 601-612.

Fryxell, PA (1965). A revision of the Australian species of *Gossypium* with observation on the occurrence of *Thespesia* in Australia. *Aust. J. Bot.* **13**, 71-102.

Louant BP, Maréchal R (1975). Comportement méiotique des hybrides trispécifiques (*Gossypium thurberi* Tod. × *G. anomalum* Wawr.) doublé × *G. hirsutum* L. et (*G. hirsutum* L. × *G. anomalum* Wavr.) doublé × *G. harknesii* Brangd. *Coton Fibres Trop.* **30**, 383 - 387.

Mergeai, G. (1994). Interspecific hybridization for cotton improvement. *In* "Cotton biotechnology" (C. Peeters ed.), pp. 23-28. FAO regional office for Europe. Technical series 32. FAO, Rome.

Mergeai G, Vroh Bi I, du Jardin P, Baudoin JP (1995). Introgression of glanded-plant and glandless-seed trait from *G. sturtianum* Willis into tetraploid cotton plants. *In* "Proceedings of the Beltwide cotton improvement conference, San Antonio (USA), 6-7 January 1995" (National Cotton Council of America ed.), pp. 513-514.

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

Stewart JMcD, Hsu CL (1977). In-ovulo embryo culture and seedling development of cotton (*Gossypium hirsutum* L.). *Planta* **137**, 113-117.

(9 ref.)