Plant Breeding **126**, 176—181 (2007) doi:10.1111/j.1439-0523.2007.01325.x

Cytogenetics of a new trispecies hybrid in cotton: [(*Gossypium hirsutum* L. × *G. thurberi* Tod.)^2 × *G. longicalyx* Hutch. & Lee]

O. N. Konan[1], A. D'Hont[2], J.-P. Baudoin[1] and G. Mergeai[1,3]

[1]Unité de Phytotechnie tropicale et d'Horticulture, Faculté Universitaire des Sciences agronomiques de Gembloux, 2 Passage des Déportés, B-5030 Gembloux, Belgium; [2]CIRAD, Centre International en Recherche Agronomique pour le Développement, UMR1096, TA 70/03, Avenue Agropolis, F-34398 Montpellier Cedex 5, France; [3]Corresponding author, E-mail: mergeai.g@fsagx.ac.be

With 5 figures and 3 tables

Received May 8, 2006/Accepted August 7, 2006
Communicated by A. M. Chevre

Abstract

A three-species hybrid named HTL including *Gossypium hirsutum* L. [2n = 4 x = 52, $(AD)_1$ genome] was created using the pseudophyletic introgression method with *G. longicalyx* Hutch. & Lee (2n = 2x = 26, F_1 genome) as donor parent and *G. thurberi* Tod. (2n = 2x = 26, D_1 genome) as bridge species. The new hybrid was totally self-sterile and its interspecific status was confirmed using simple sequence repeat markers and cytogenetic analysis. Cytogenetic studies showed that its chromosome configuration was 2n = 52 = 14.13 I + 15.10 II + 1.03 III + 0.9 IV + 0.03 V + 0.13 VI (where I, II, III, IV, V and VI are univalents, bivalents, trivalents, tetravalents, pentavalents and hexavalents, respectively). Prospects for successfully exploiting the HTL hybrid in breeding programmes are discussed.

Key words: *Gossypium* spp. — interspecific hybridization — SSR markers — cytogenetic analysis

The cotton genus *Gossypium* L. (Malvaceae) contains 45 diploid species (2n = 2x = 26) and five tetraploid species (2n = 4x = 52) (Fryxell 1979, Fryxell et al. 1992). Diploid species are differentiated into eight genome groups designated by the capital letters A, B, C, D, E, F, G and K (Endrizzi et al. 1985, Stewart 1995). The sole F genome species, *G. longicalyx*, is separated from the remaining genome groups by its distinctive geography, morphology and ecology (Fryxell et al. 1992). All the above diploid *Gossypium* genomes contain exclusively wild species, except for the A genome. The tetraploid species contain two distinct subgenomes related to the A and D diploid genomes (Wendel and Cronn 2003). The tetraploid species *G. hirsutum* L., cytogenetically known as (A_hD_h) or $(AD)_1$, is the main cultivated cotton throughout the world. It accounts for 95% of the world production of lint.

All diploid genomes are important genetic resources that can contribute valuable genes for fibre quality, resistance to diseases and insect pests, tolerance to abiotic stress, and cytoplasmic genes conditioning male sterility along with nuclear restorer genes (Stewart 1995). However, only a small part of this valuable genetic diversity has been utilized through introgression into cultivated cotton (Mergeai 2004).

In the exploitation of the genetic diversity of *G. longicalyx*, two male-sterile triple-species hybrids denoted as HLA [(*G. hirsutum* × *G. longicalyx*)^2 × *G. armourianum*] and HHL [(*G. hirsutum* × *G. herbaceum*)^2 × *G. longicalyx*] were developed by A. A. Bell of the US Department of Agriculture (Robinson et al. 2004). Chromosome numbers and meiotic metaphase I configurations were assessed for the BC1F1, BC2F1, BC1S1, BC3F1, BC4F1 and BC5F1 progenies of these hybrids (Dighe et al. 2005) but no data have been published so far concerning the cytogenetics of the HLA and HHL triple-hybrids themselves.

The success of an interspecific breeding programme is dependent upon two factors. First, a fertile hybrid must be developed involving the donor and the recipient genotype, directly or through the creation of an intermediate bridge structure, to allow the union into a single nucleus of the donor and the recipient genotype chromosomes. Secondly, genetic recombination between the donor and recipient chromosomes must occur. The degree to which these two events happen depends on the genetic compatibility between the donor and recipient species. In such interspecific breeding programmes, cytogenetic studies allow confirmation of the karyologic constitution of the hybrids produced and thereby facilitate an assessment of the chances of their successful exploitation.

In this article, the results of the cytogenetic analysis of the trispecies hybrid [(*G. hirsutum* × *G. thurberi*)^2 × *G. longicalyx*] are presented.

Materials and Methods

Plant materials: At Gembloux Agricultural University, the fertile (*Gossypium hirsutum* × *G. thurberi*)^2 hexaploid [2n = 6x = 78, $2(A_hD_hD_1)$] was crossed to the wild diploid species *G. longicalyx* Hutch. and Lee (2n = 2x = 26, $2F_1$) to create the trispecies hybrid [(*G. hirsutum* × *G. thurberi*)^2 × *G. longicalyx*] [2n = 4x = 52, $2(A_hD_hD_1F_1)$] using the pseudophyletic introgression method (Mergeai 2004). This method ends at the creation of trispecific hybrids involving *G. hirsutum* and two diploid species. *Gossypium hirsutum* is crossed directly with one of the diploid parents, creating a triploid hybrid. Chromosome doubling of the hybrid gives a fertile allohexaploid which is crossed to the other diploid species, resulting in the researched allotetraploid trispecific hybrid. In the present case, the triple-species hybrid obtained was denoted as HTL. The crossing scheme followed is shown in Fig. 1. Emasculation, pollination and growth regulator application techniques were achieved as follows: flowers were emasculated in the afternoon before anthesis and the stigma was covered by a small plastic sachet; pollen was applied to stigmas between 08:00 and 11:00 h the following morning. To avoid capsule shedding, a small piece of cotton wool containing a drop of the growth regulator solution (100 mg l^{-1} naphtoxyacetic acid + 50 mg l^{-1} gibberellic acid) recommended by Altman (1988) was applied on the ovary just after pollination.

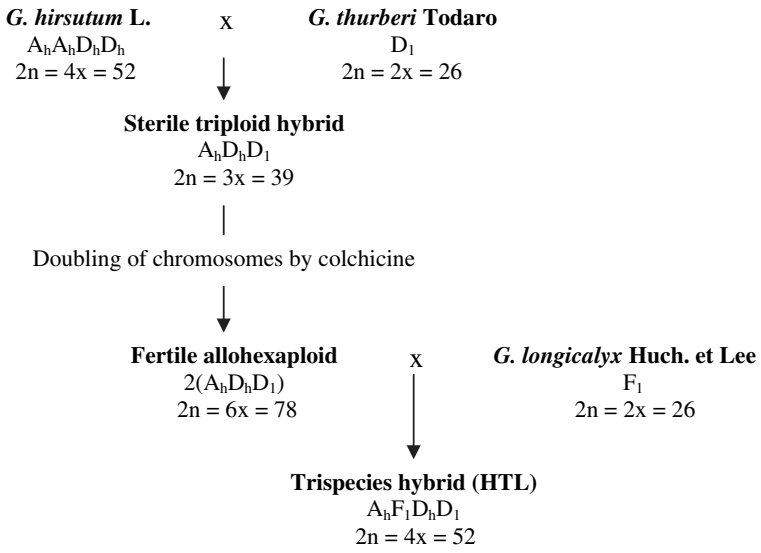

Fig. 1: Development scheme of the trispecies hybrid [(*G. hirsutum* × *G. thurberi*)^2 × *G. longicalyx*]

Microsatellite markers (SSR analysis): Fifteen microsatellite markers (SSR) developed at Brookhaven National Laboratory (BNL 1897, BNL 3989, BNL 4030, BNL 2448, BNL 3029, BNL 3992, BNL 1440, BNL 1417, BNL 3436, BNL 1604, BNL 2847, BNL 256, BNL 1017, BNL 3556 and BNL 2961) were used to verify the hybridity of the material produced. Clone sequences used for primer definition are available at http://ukcrop.net/perl/ace/search/CottonDB. These SSR markers showed a polymorphism between the three parental species of the HTL hybrid and they were distributed on chromosomes c2, c3, c5, c6, c7, c9, c10, A02, D08 and D03 of *G. hirsutum*. Protocols followed to achieve the DNA extraction and the SSR analyses were those of Murray and Thompson (1980) modified by Vroh Bi et al. (1996) and of Liu et al. (2000).

Mitotic chromosome preparations: To check the chromosome numbers of the hybrids obtained, mitotic chromosome preparations were carried out using root tips. Roots were harvested from seeds germinated in Petri dishes placed in a steam room at 30°C and from plants cultivated in pots. They were treated in 0.04% hydroxyquinoline for 4 h in the dark at room temperature, fixed for 48 h in Carnoy's I fluid (glacial acetic acid : ethanol, 1 : 3) and stored in 70% ethanol at 4°C. Chromosome preparations were made according to the method described by D'Hont et al. (1995). After staining with 4,6-diamino-2-phenylindole/Vectashield, chromosomes were visualized and counted under fluorescent light with a microscope Nikon Eclipse E800 (Nikon, Tokyo, Japan) equipped with a JVC KY-F 58E camera (JVC, Yokohama, Japan).

Meiotic chromosome preparations: For chromosome association analysis, suitable flower buds were collected between 09:00 and 11:00 h, according to the weather conditions, and fixed in fresh Carnoy's II fluid (glacial acetic acid : chloroform : ethanol, 1 : 3 : 6) for 48–72 h. They were then stored at 4°C in 70% ethanol until their evaluation. To obtain meiotic plates, a few anthers were squashed in a drop of 1.5% acetocarmine solution on a microscope slide, debris was removed, and the slide was covered with a coverslip and heated a few seconds over a flame to improve chromosome staining. With pressure on the coverslip, pollen mother cells were flattened to spread out chromosomes. Observations were made with a Nikon Eclipse E800 photomicroscope (Nikon) under oil immersion.

Pollen fertility: To analyse pollen fertility, flowers were collected in the morning on the day of anthesis. About 200 pollen grains per plant were dipped in 1.5% acetocarmine solution for 30 min and pollen fertility was estimated under a stereomicroscope as the percentage of stained pollen; only large, bright and red grains were considered fertile.

Results

Hybrid production and hybridity confirmation

From 34 crosses achieved between the hexaploid (*G. hirsutum* × *G. thurberi*)^2 and *G. longicalyx*, 10 seeds were obtained. Nine of these gave viable plants from which informative SSR markers were used to check the hybridity of the material produced. For all 15 SSR markers tested, the hexaploid exhibited SSR bands of both the parental species *G. hirsutum* and *G. thurberi* while the HTL hybrid plants showed SSR bands of the three parental species *G hirsutum*, *G. thurberi* and *G. longicalyx* (Fig. 2). These results confirm the success of the crosses achieved and the trispecies hybridity of the material produced.

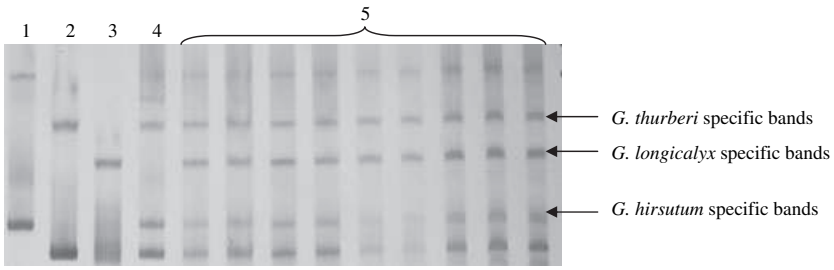

Fig. 2: SSR electrophoresis profile of the primer BNL 4030 showing the triple species character of the hybrid [(*G. hirsutum* × *G. thurberi*)^2 × *G. longicalyx*]: (1) *G. hirsutum*; (2) *G. thurberi*; (3) *G. longicalyx*; (4) hexaploid (*G. hirsutum* × *G. thurberi*)^2; (5) nine plants of the hybrid [(*G. hirsutum* × *G. thurberi*)^2 × *G. longicalyx*]

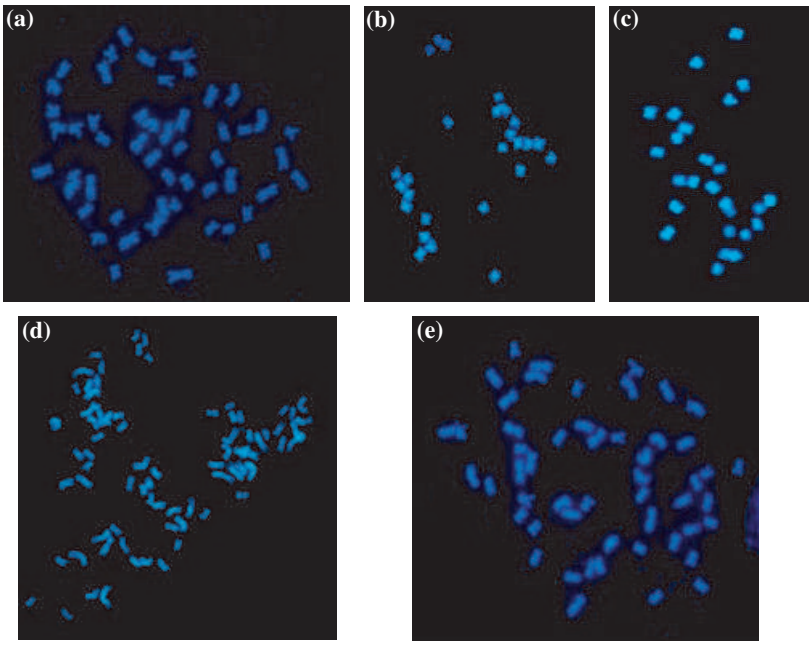

Fig. 3: Chromosomal configuration at somatic metaphase: (a) 52 chromosomes of *G. hirsutum* (×1500); (b) 26 chromosomes of *G. longicalyx* (×1000); (c) 26 chromosomes of *G. thurberi* (×1000); (d) 78 chromosomes of the (*G. hirsutum* × *G. thurberi*)^2 hexaploid (×600); (e) 52 chromosomes of the [(*G. hirsutum* × *G. thurberi*)^2 × *G. longicalyx*] trispecies hybrid (×1250)

Mitotic chromosome analysis

Analysis of mitotic metaphase plates showed 52 chromosomes for *G. hirsutum*, 26 chromosomes for *G. thurberi*, 26 chromosomes for *G. longicalyx*, 78 chromosomes for the (*G. hirsutum* × *G. thurberi*)^2 hexaploid and 52 chromosomes for the HTL hybrid (Fig. 3). The HTL hybrid has a chromosome number similar to that of *G. hirsutum*. This result confirms the hybrid status of the material obtained because it agrees with the number of chromosomes expected for a trispecific hybrid issued from crossing a hexaploid with a diploid.

Meiotic chromosome associations

Meiotic metaphase I plates of *G. hirsutum* show 26 bivalents (Fig. 4a). The 52 chromosomes of *G. hirsutum* pair perfectly. Cytological data for the F1 HTL trispecies hybrid [(*G. hirsutum* × *G. thurberi*)^2 × *G. longicalyx*] are summarized in Table 1. Chromosome associations in the HTL hybrid were variable, with univalents, bivalents and multivalents (Fig. 4b). The examination of 30 metaphase plates of this hybrid shows a mean of 15.10 bivalents. The number of bivalents ranges from 9 to 22, with 14 being the most frequent number. The number of univalents ranges from 5 to 26, with an average of 14.13. The most frequent number is 12. One or more multivalents, with a maximum of four, may be present, most often as trivalents and quadrivalents, rarely as pentavalents or hexavalents. The average numbers of multivalents are 1.03 III, 0.9 IV, 0.03 V and 0.13 VI. On average, 37.82 chromosomes of the 52 HTL chromosomes paired at metaphase I.

Pollen fertility

The pollen grains of the parental species *G. hirsutum*, *G. thurberi* and *G. longicalyx* have a uniform size and are easily stainable with acetocarmine (Fig. 5a). Pollen stainability of all parental species is about 100% (Table 2), indicative of the fertility of these species. For the hexaploid (*G. hirsutum* × *G. thurberi*)^2 and the trispecies hybrid HTL, pollen grain size was not uniform and variation in stainability was observed (Fig. 5b). The mean proportion of pollen grains stainable with acetocarmine was 42.57% for the hexaploid and 9.03% for the HTL trispecies hybrid (Table 2). With 42.57% pollen fertility the (*G. hirsutum* × *G. thurberi*)^2 hexaploid produced capsules with viable seeds while the HTL hybrid did not produce any mature capsules. All HTL capsules aborted about 2–3 days after anthesis. The HTL trispecies hybrid seems to be completely self-sterile. This sterility is in line with the low percentage of stainable pollen grains observed and the irregular meiotic pairing.

Discussion

Important differences were observed in meiotic metaphase I plates between the tetraploid cotton *G. hirsutum* and the trispecies hybrid [(*G. hirsutum* × *G. thurberi*)^2 × *G. longicalyx*]. While all 52 chromosomes of *G. hirsutum* paired perfectly in 26 bivalents, the HTL trispecies hybrid exhibited a mean of 14.13 univalents and some multivalents. The univalents observed represent chromosomes that did not find homologous to associate with in the trispecies hybrid. This lack of homology could be attributed either to accumulations of chromosomal modifications during *Gossypium* genome differentiation or to asynapsis genes with specific effects on chiasma formation (Kammacher 1956). As the D_h subgenomes of *G. hirsutum* retain sufficient chromosome homology with species of the D genome group (Brown and Menzel 1952, Endrizzi 1957, Phillips and Strickland 1966), the unpaired chromosomes noted here should be mostly those of non-homologous chromosomes of the *G. longicalyx* F_1 genome and the *G. hirsutum* A_h subgenome.

The multivalents observed in the current study may be due to segmental interchanges that occurred in *Gossypium* during the evolution of this genus (Kammacher 1966, Maréchal 1974, Vroh Bi et al. 1998). In such complex hybrids with chromosomes coming from different genomes, residual homology between some chromosomes that carry these interchanged segments can lead to multivalents. Bivalents and multivalents, by their chiasma, represent good prospects for chromosome

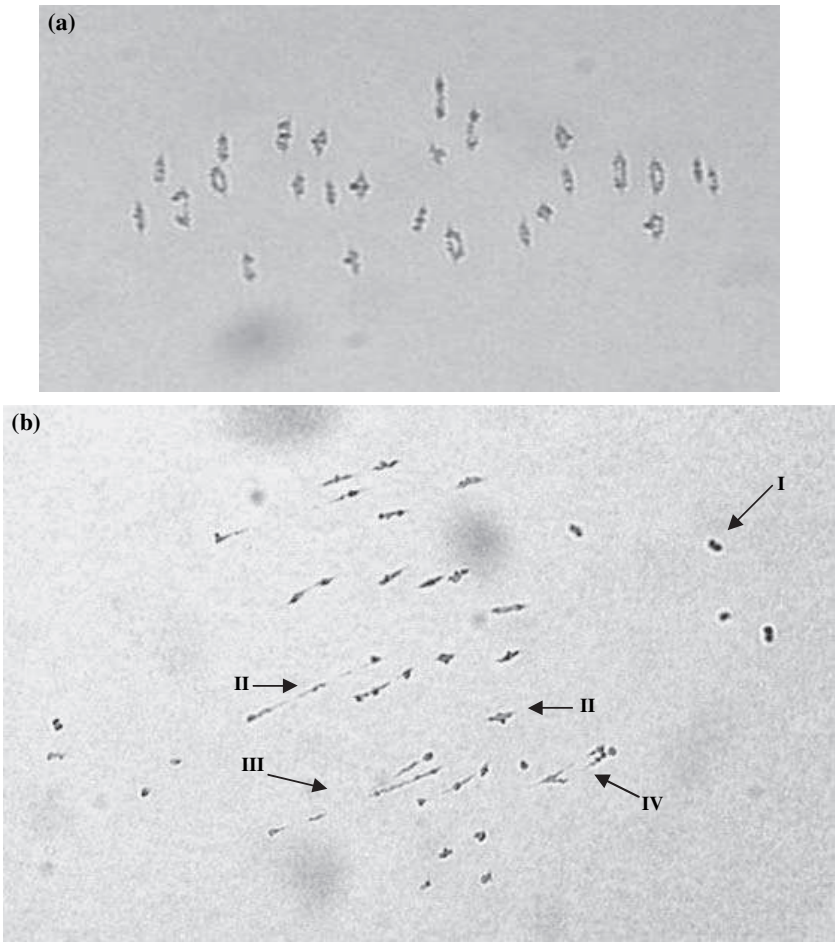

Fig. 4: Meiotic metaphase I plates: (a) *G. hirsutum* ($A_hA_hD_hD_h$, $2n = 2x = 52$): 26 bivalents (×600); (b) [(*G. hirsutum* × *G. thurberi*)^2 × *G. longicalyx*] trispecies hybrid ($A_hD_hD_1F_1$, $2n = 4x = 52$): 15I, 14II, 1III, 1VI (×600)

recombination. This phenomenon is important for introgression of desired traits.

The self-sterility of the HTL trispecies hybrid may be due to the univalents and multivalents observed. The presence of these chromosome associations can provoke abnormal separation of the chromosomes during the first meiotic division and generate unbalanced gametes with heterogeneous chromosome numbers leading to sterility (Kammacher 1956). The multivalents could be more responsible for the sterility than the univalents. Indeed, the [(*G. hirsutum* × *G. arboreum*)^2 × *G. raimondii*] ($A_hA_2D_hD_1$) trispecies hybrid, despite its low univalent number (owing to the close affinity between *G. hirsutum* and the diploid parents), was self-sterile (Kammacher 1966), like the HTL hybrid. According to Kammacher (1966), the presence of a hexavalent decreases ovule fecundity by 50% and pollen fertility by 15%; a tetravalent does not affect ovule fecundity but divides pollen fertility by two; the simultaneous presence of these chromosome associations in the metaphasic plates of a particular hybrid decreases by 50% its female fertility and induces insignificant pollen fertility. The rather high level of male sterility observed in the HTL trispecies hybrid implies that this hybrid should preferably be used as the female parent when backcrossed to *G. hirsutum* cultivars.

The comparison of the chromosome configurations observed in HTL and in other cotton trispecific hybrids mentioned in the literature (Table 3) confirms the soundness of the mating scheme followed to exploit the genetic diversity of *G. longicalyx*. The few univalents observed in $A_hA_2D_hD_1$, $A_hA_2D_hD_{2-2}$ and $A_hA_1D_hD_{2-2}$ trispecific hybrids are mainly due to the reciprocal translocations existing between the A_h subgenome and A_1 (*G. herbaceum*) and A_2 (*G. arboreum*) (Brown and Menzel 1950) while the number of univalents in the other trispecific hybrids may be attributed to an interaction between the diploidization factor existing in *G. hirsutum* and the pairing affinities characterizing the chromosomes of the non-*hirsutum* genomes that are found in the trispecific structures (Louant and Maréchal 1975). Because of the use of a D-genome bridge species, the number of univalents observed in the HTL hybrid is rather low (only about 25% of its 52 chromosomes) and similar to that noted in the HRS and HTS hybrids that were successfully backcrossed to *G. hirsutum* cultivar by Vroh Bi et al. (1999). As female gametes tolerate multiple alien chromosome addition and alien chromosome fragment introgression in their nucleus better than pollen, fertile progenies were produced by backcrossing most of the trispecific hybrids presented in Table 3 to *G. hirsutum* cultivars. In general, progression towards 100% 52-chromosome individuals and increasing frequencies of plants that form 26 bivalents at metaphase I were observed in the advanced backcross generations of all these hybrids. It was also the case for the HHL [(*G. hirsutum* × *G. herbaceum*)^2 × *G. longicalyx*] and HLA [(*G. hirsutum* × *G. longicalyx*)^2 × *G. armourianum*] male-sterile trispecific hybrids (Dighe et al. 2005), the latter having a genomic constitution very close to the present HTL hybrid (they used *G. armourianum* instead of *G. thurberi* as bridge species).

Table 1: Meiotic pairing in the [(*G. hirsutum* × *G. thurberi*)^2 × *G. longicalyx*] trispecies hybrid ($A_hD_hD_1F_1$)

Plate no.	Chromosome configuration I	II	III	IV	V	VI	Chromosome no.
1	15	17	1				52
2	5	22	1				52
3	20	14		1			52
4	22	12	2				52
5	12	13	2	2			52
6	17	16	1				52
7	14	19					52
8	18	15		1			52
9	15	15	1	1			52
10	10	14	2	2			52
11	12	13	2	2			52
12	14	14		1		1	52
13	16	13		1		1	52
14	17	11	3	1			52
15	9	18	1	1			52
16	10	15	4				52
17	26	11		1			52
18	13	16	1	1			52
19	15	9	1	4			52
20	26	13					52
21	11	13	2	1	1		52
22	12	14		3			52
23	13	15	3				52
24	10	16	2	1			52
25	8	18		2			52
26	8	20		1			52
27	7	21	1				52
28	12	20					52
29	16	12				2	52
30	21	14	1				52
Min.–max.	5–26	9–22	0–4	0–4	0–1	0–2	52
Mean	14.13	15.10	1.03	0.9	0.03	0.13	52

Average number of chromosomes paired: 37.82.

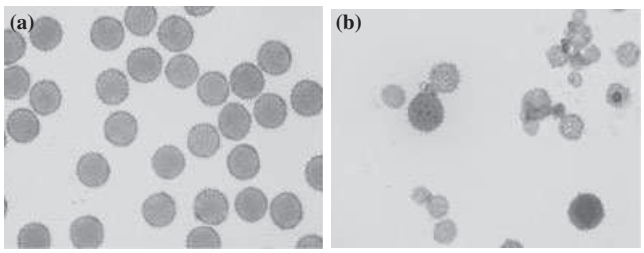

Fig. 5: Cotton pollen grain fertility revealed by acetocarmine staining: (a) *G. hirsutum* pollen grains with good colour and uniform size (×200); (b) pollen grains of [(*G. hirsutum* × *G. thurberi*)^2 × *G. longicalyx*] trispecies hybrid with variation in colour and in size (×200)

Table 2: Pollen fertility of the [(*G. hirsutum* × *G. thurberi*)^2 × *G. longicalyx*] trispecies hybrid and of its parents

Genotypes	No. pollen grains Fertile	Sterile	Total	Pollen fertility rate (%)
HTL hybrid	215	2166	2381	9.03
G. hirsutum (C2)	200	0	200	100.00
G. thurberi	131	0	131	100.00
G. longicalyx	200	0	200	100.00
(*G. hirsutum* × *G. thurberi*)^2 hexaploid	129	174	303	42.57

Table 3: Comparison of the chromosome (Chr.) configurations of the different trispecific hybrids created in *Gossypium*

Hybrid combinations	Chr. no.	Chr. configuration I	II	III	IV	V	VI	VII	VIII	IX	X	No. observed cells	References
[(*G. arboreum* × *G. thurberi*)^2 × *G. hirsutum*] ($A_hA_2D_hD_1$)	52	0.88	20.93	0.62	1.01	0.14	0.27	0.00	0.02	0.00	0.02	58	Brown and Menzel (1950)
[(*G. hirsutum* × *G. arboreum*)^2 × *G. harknessii*] ($A_hA_2D_hD_{2-2}$)	52	1.37	22.36	0.31	1.09	0.01	0.08	0.00	0.00	0.00	0. 00	37	Brown and Menzel (1950)
[(*G. hirsutum* × *G. herbaceum*)^2 × *G. harknessii*] ($A_hA_1D_hD_{2-2}$)	52	1.84	22.14	1.14	0.54	0.00	0.05	0.00	0.00	0.00	0.00	67	Brown and Menzel (1950)
[(*G. thurberi* × *G. anomalum*)^2 × *G. hirsutum*] ($A_hB_1D_hD_1$)	52	11.45	17.13	1.22	0.50	0.04	0.03	0.00	0.00	0.00	0.00	130	Louant and Maréchal (1975)
[(*G. hirsutum* × *G. anomalum*)^2 × *G. harknessii*] ($A_hB_1D_hD_{2-2}$)	52	17.72	16.24	0.46	0.10	0.00	0.00	0.00	0.00	0.00	0.00	90	Louant and Maréchal (1975)
[(*G. hirsutum* × *G. raimondii*)^2 × *G. sturtianum*] ($A_hC_1D_hD_5$)	52	13.64	17.04	0.85	0.35	0.00	0.07	0.00	0.00	0.00	0.00	56	Vroh Bi et al. (1999)
[(*G. thurberi* × *G. sturtianum*)^2 × *G. hirsutum*] ($A_hC_1D_hD_1$)	52	14.55	15.68	0.91	0.35	0.00	0.25	0.00	0.00	0.00	0.00	56	Vroh Bi et al. (1999)
[(*G. arboreum* × *G. bickii*)^2 × *G. hirsutum*] (A_h $A_2D_hG_1$)	52	41.04	4.54	0.57	0.04	0.00	0.00	0.00	0.00	0.00	0.00	95	Shuijin and Biling (1993)
[(*G. hirsutum* × *G. thurberi*)^2 × *G. longicalyx*] (A_h $F_1D_hD_1$)	52	14.13	15.10	1.03	0.9	0.03	0.13	0.00	0.00	0.00	0.00	30	Konan et al. (this study)

These data indicate that there are good chances of success for the future exploitation of the HTL hybrid in cotton breeding programmes.

Acknowledgements

This research was financially supported by Dow Agrosciences LLC. The PhD grant of the first author was funded by the Government of Ivory Coast.

References

Altman, D. W., 1988: Exogenous hormone applications at pollination for in vitro and in vivo production of cotton interspecific hybrids. Plant Cell Rep. **7,** 257—261.

Brown, M. S., and M. Y. Menzel, 1950: New trispecies hybrids in cotton. J. Hered. **41,** 291—295.

Brown, M. S., and M. Y. Menzel, 1952: The cytology and crossing behavior of *Gossypium gossypioides*. Bull. Torrey Bot. Club **79,** 110—125.

D'Hont, A., P. S. Rao, P. Feldmann, and L. Grivet, 1995: Identification and characterisation of sugarcane intergeneric hybrids, *Saccharum officinarum* × *Erianthus arundinaceus*, with molecular markers and DNA in situ hybridisation. Theor. Appl. Genet. **91,** 320—326.

Dighe, N. D., D. M. Stelly, M. Menz, A. F. Robinson, A. A. Bell, and A. Bridges, 2005: Reniform nematode resistance – cytogenetics of *Gossypium longicalyx* introgression products. In: P. Dugger, and D. Richter (eds), Proc. Beltwide Cotton Conf. New Orleans, LA January 4–7, 2005, 194. National Cotton Council, Memphis, TN [abstract].

Endrizzi, J. E., 1957: The cytology of two hybrids of *Gossypium*. J. Hered. **48,** 221—226.

Endrizzi, J. E., E. L. Turcotte, and R. J. Kohel, 1985: Genetics, cytology, and evolution of *Gossypium*. Adv. Genet. **23,** 271—375.

Fryxell, P. A., 1979: The Natural History of the Cotton Tribe. Texas A&M University Press, College Station, TX.

Fryxell, P. A., L. A. Craven, and J. M. Stewart, 1992: A revision of *Gossypium* sect. *Gandicalyx* (Malvaceae), including the description of six new species. Syst. Bot. **17,** 91—114.

Kammacher, P., 1956: Les possibilités actuelles d'application de l'hybridation interspécifique à l'amélioration du cotonnier en milieu africain. Coton et Fibres Tropicales **11,** 101—136.

Kammacher, P., 1966: Etude des relations génétiques et caryologiques entre génomes voisins du genre *Gossypium*. Coton et Fibres Tropicales **21,** 263—290.

Liu, S., R. G. Cantrell, J. C. McCarty, and J. McD. Stewart, 2000: Simple sequence repeat-based assessment of genetic diversity in cotton race stock accessions. Crop Sci. **40,** 1459—1469.

Louant, B. P., and R. Maréchal, 1975: Comportement méiotique des hybrides trispécifiques (*Gossypium thurberi* Tod. × *G. anomalum* Wawr.) doublé × *G. hirsutum* L. et (*G. hirsutum* L. × *G. anomalum* Wawr.) doublé × *G. harknessii* Brandg. Coton et Fibres Tropicales **30,** 383—387.

Maréchal, R., 1974: Analyses de la conjugaison méoitique chez les hybrides triploïdes entre *Gossypium hirsutum* L. et des espèces sauvages australiennes. Bull. Rech. Agron. Gemboux **9,** 193—204.

Mergeai, G., 2004: Forty years of genetic improvement of cotton through interspecific hybridisation at Gembloux Agricultural University: achievement and prospects. In: A. Swanepoel (ed.), Proc. World Cotton Research Conference – 3. Cotton Production for the New Millennium. Cape Town, South Africa, 9–13 March 2003, 120—133. Agricultural Research Council, Institute for Industrial Crops, Pretoria.

Murray, M., and W. F. Thompson, 1980: Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. **8,** 4321—4325.

Phillips, L. L., and M. A. Strickland, 1966: The cytology of a hybrid between *Gossypium hirsutum* and *G. longicalyx*. Can. J. Genet. Cytol. **8,** 91—95.

Robinson, A. F., A. A. Bell, N. Dinghe, and D. Stelly, 2004: Status report on introgression of reniform nematode resistance from *Gossypium longicalyx*. In: P. Dugger and D. Richter (eds), Proc. Beltwide Cotton Conf. San Antonio, TX, USA, January 5–9, 2004. 1119. National Cotton Council, Memphis, TN.

Stewart, J. M., 1995: Potential for crop improvement with exotic germplasm and genetic engineering. In: G. A. Constable and N. W. Forrester (eds), Proc. World Cotton Research Conference-1. Challenging the Future. Brisbane, Australia, February 14–17, 1994, 313—327. CSIRO, Melbourne.

Vroh Bi, I., A. Chandelier, G. Mergeai, and P. Du Jardin, 1996: Improved RAPD amplification of recalcitrant plant DNA by the use of activated charcoal during DNA extraction. Plant Breeding **115,** 205—206.

Vroh Bi, I., J. P. Baudoin, and G. Mergeai, 1998: Cytogenetics of cotton plants used to introgress the "glandless seed-glanded plant" trait from *Gossypium sturtianum* Willis into cultivated upland cotton (*Gossypium hirsutum* L.). Plant Breeding **117,** 235—241.

Vroh Bi, I., J. P. Baudoin, B. Hau, and G. Mergeai, 1999: Development of high-gossypol cotton plants with low gossypol seeds using trispecies bridge crosses and in vitro culture of seed embryos. Euphytica **106,** 243—251.

Wendel, J. F., and R. C. Cronn, 2003: Polyploidy and the evolutionary history of cotton. Adv. Agron. **78,** 139—186.

Shuijin, Z., and L. Biling, 1993: Studies of introgression of the "glandless seeds-glanded plant" trait from *Gossypium bickii* into cultivated upland cotton (*G. hirsutum*). Cotton Fibres Trop **43,** 195—199.