Isolation and characterisation of seven alien monosomic addition lines of *Gossypium australe* F. Muell. on *G. hirsutum* L.

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ABSTRACT

*The hexaploid 2(*Gossypium hirsutum *L. -x* G. australe *F.- Muell.) was backcrossed to G. hirsutum to produce the pentaploid G.- hirsutum x G.- australe and seven alien monosomic addition lines of Gossypium australe on G. hirsutum. The different hybrids produced were characterized morphologically, cytogenetically and using genomic in situ hybridization and eighty-six Simple Sequence Repeat (SSR) markers. The analysis of their progenies allowed to quantify the frequency of genetic material exchanges between the supernumerary alien chromosome and G . hirsutum. The seven monosomic addition lines obtained constitute valuable genetic stocks to carry out fundamental and applied investigations.*

Introduction

The wild diploid *Gossypium australe* F. Muell. (2n=2x=26, G_2 genome) is indigenous to the Australian continent. While the species is unacceptable for commercial fiber production, *G. australe* is susceptible to bring several desirable traits that are absent in the primary gene pool of the main cultivated cotton species, *G. hirsutum* L. These traits include (i) glandless seed but a glanded plant, (ii) drought resistance, (iii) resistance to green aphid and mites, (iv) improved fibre ginning percentage and fibre maturity (Demol *et al., 1*978, Ndungo *et al.*,1988; Brubaker *et al.*,1996). The production of monosomic addition lines from interpecific hybrids developed through the aphyletic introgression method is an efficient way to enhance gene transfer from diploid donor cotton species to *G. hirsutum* (Hau 1981, Mergeai, see these proceedings). In addition to enhanced gene transfer, alien chromosome addition lines provide a means of distinguishing the effect of specific alien chromosomes and detecting homeologies with chromosomes of cultivated species (Rooney *et al., 1*991). Our objectives were to isolate and characterize as many as possible monosomic addition stocks in the progeny of the *G. hirsutum* x *G. australe* allohexaploid hybrid.

Experimental procedure

Plant material

First (6x/1)[1] and second (6x/2)[1] generation *G. hirsutum* x *G. australe* hexaploids (G411[2], G430[2]) from Gembloux Agricultural University (GAU) cotton collection (Maréchal, 1983) created according to the aphyletic introgression method (Mergeai, see these proceedings) were backcrossed at Gembloux, Belgium in 1998 and 1999 to *G. hirsutum* cultivar Stamf originating from Togo (West Africa) to produce BC_1 pentaploid derivatives. The G411² and G430² *G. hirsutum* x *G. australe* hexaploids contain the genome of *G. australe* accession G319² and of *G. hirsutum* cv. NC8 (G173²) originating from the Democratic Republic of Congo. The first pentaploids obtained from backcrossing these hexaploids to cv. Stamf were either selfed or backcrossed as male and female parent to Stamf to produce BC_1S_1 and BC_2 seeds at Gembloux in 1999. The BC_1Pentaploids and a portion of the BC_1S_1 and BC_2 hexaploid created in Belgium were grown at Cotonou, Republic of Benin, West Africa, from November 1999 to April 2000 to produce BC_1S_1, BC_1S_2, BC_2, and BC_2S_1 materials. A portion of some of the BC_2S_1 progenies from *G. hirsutum* x *G. australe* hexaploids were cultivated at Gembloux in 2000 and 2001. In Belgium, the new materials obtained in the framework of this work were planted each year in early May and cultivated year-round in glasshouses under native light conditions. In Cotonou, plants were cultivated in field conditions. The backcrossing scheme was accomplished in the following manner. Flowers were emasculated the afternoon before anthesis and the stigma was covered ~~by~~ with a small plastic sachet. Pollen was applied to stigmas between 08:00 and 11:00 hours the following morninG. ~~To avoid capsule shedding,~~ Aa 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 *et al.* (1988), was applied on the ovary just after pollination to avoid capsule shedding. Self pollination were forced– by clipping the flower bud at candle stage. Hybridizsation results were pooled by hybrid type to facilitate their interpretation and because no substantial variation among accessions of a same hybrid formula was evident.

Mis en forme

Pollen fertility assessment

Pollen grain fertility was assessed in Gembloux according to two methods, acetocarmine staining (15 g carmine per ~~litre~~liter of acetic acid) and the germination method proposed by Barrow (1981). For both methods, 1000 pollen grains produced from two freshly opened flowers were used. Only large, bright red grains were considered fertile when observed after 30 minutes in acetocarmine solution. Any evidence of pollen tube growth was used to identify fertile pollen grains.

Assessment of the gossypol content of the seeds

The gossypol content was assessed seed by seed on samples of 20 seeds using the model developed by Benbouza *et al.* (2002~~) :~~): $\%G = b_i\ (N/S)$; where $\%G$ is the content of gossypol in %, N is the number of gossypol glands per seed section, S is the area of the seed section expressed in mm^2, and b_i is a regression coefficient depending on the considered genotype. To obtain the data needed to calculate the seed gossypol content, each seed was cut in two longitudinal sections after removal of the teguments in order to assess its total number of glands N per section and its section area S in mm². These operations were carried out with a Nikon Eclipse E800 light and fluorescent microscope (Nikon, Tokyo, Japan) using a JVC-3-CCD ~~colour~~color video

[1] (6x/1), (6x/2~~) :~~): first and second generation of hexaploid after chromosome doubling
[2] Accession numbers of seed stock in the GAU cotton collection

camera (JVC, Tokyo, Japan) and the Archive Plus program of Sony (Sony Electronics, NJ, Park Ridge, USA) to capture and ~~analyse~~analyze the images.

Cytogenetic analysis

Young flower buds were collected between 08:00 and 11:00 according to weather conditions and fixed in fresh Carnoy solution (95% ethanol – chloroform – glacial acetic acid, 6:3:1, v:v:v). The fixing solution was replaced by 70% ethanol after 48 to 72 h and the buds stored at 4°C until evaluated. Metaphase I squashes were obtained by macerating and grinding with a scalpel a few anthers in a drop of acetocarmine solution on a microscope slide, removing the debris, adding a ~~coverslip~~cover slip, differentiating the chromosomes with mild heat and flattening pollen mother cells with pressure on the ~~coverslip~~cover slip. Observations were made with a Nikon Eclipse E800 photomicroscope (Nikon, Tokyo, Japan) under oil immersion.

In situ hybridization

Chromosome preparation of the hexaploid was made from germinated seeds (on moist filter paper in Petri dishes for 72 h at 30°C) in CIRAD, while root tips were excised directly from adult monosomic addition plants in Gembloux. All roots excised were treated with 0.04% 8-hydroxyquinoline at room temperature for 4 h, fixed for 48h to 72h in 3:1 ethanol:acetic acid solution at room temperature and stored in 70% at 4°C. Metaphase spreads were prepared as described previously by D'hont et al (1996). Fixed root-tips were digested with cellulase and pectolyase before spreading on a slide with a drop of 3:1 ethanol : acetic acid solution. The preparations were screened using contrast microscopy and selected slides were used immediately or stored at –70°C before *in situ* hybridization. Total genomic DNA from G. hirsutum cultivar Stamf was labeled with digoxigenin 11-dUTP by nick translation (Kit Boehringer-Manheim 174516); total genomic DNA from G. australe was labeled with biotine 14-dUTP by nick translation (Kit Gibco BRL 18247-015). Chromosomes were examined under epifluorescence microscope. The images were captured with a CDD camera and the QFISH Leica software.

SSR analysis

For all the plants analyzed with SSR markers, DNA extractions were carried out in Gembloux using the protocol developed by Vroh ~~bi~~ B *et al.* (1996), while SSR reactions were carried out in CIRAD. The simple sequence repeat (SSR) markers used to characterize the hexaploid *G. hirsutum* x *G. australe* (G411), its parents, and part of its BC_2S_1 progeny were derived from a repeat-enriched cotton genomic library developed by B. Burr at Brookhaven National Laboratory. Clone sequences used for primer construction are available at http://demeter.bio.bnl.gov/acecot.html. The SSR analysis conditions were as described in Risterucci *et al.* (2000), using a 5' end ~~labelling~~labeling of the forward primer with γ-[^{33}P] ATP and a 55°C annealing temperature. The SSR reported were initially chosen for their ability to yield polymorphic PCR products between the two parents of a 'Guazuncho 2' (*G. hirsutum*) x 'VH8' (*G. barbadense*) BC_1F_1 population, as well as for their mapping position on the tetraploid genetic map (Lacape *et al.*, 2003). Each of the thirteen pairs of homoeologous A and D chromosomes of the map were represented by a minimum of three SSRs. Totally, 86 SSRs were tested on 20 DNAs including the 6x/1 *G. hirsutum* x *G. australe* hexaploid G411, 13 monosomic

addition lines of *G. australe* on *G. hirsutum* isolated in G411 BC_2 progeny, *G australe* accession G319, as well as C2, NC8, and Stam F, and two BC_2S_1 plants carrying 25 bivalents and two univalents.

Results

BC_1, BC_2 and BC_1S_1 materials were produced rather easily from the *G. hirsutum* x *G. australe* hexaploids. The percentages of stainable and geminated pollen grains of the hexaploid were respectively 37.2 and 42.8 %. On an average, 1.6 BC1 seeds per pollination were produced by backcrossing the hexaploids to *G. hirsutum* Cv. Stamf. The *G. hirsutum* x *G. australe* BC_1 pentaploids had 15.6% of pollen grains stained and a pollen grain germination rate of 19.7%. The best success rate for the production of BC_2 seeds (1.3 BC_2 seeds per pollination) was obtained when using the BC_1 pentaploid as male parent while the reverse crosses and the selfing of the pentaploids gave much lower results (respectively 0.27 BC_2 and 0.1 BC_1S_1 seeds per pollination). Among the BC_2 and BC_1S_1 progeny of the allohexaploids, 310 out of the 311 self fertile plants obtained were BC_2 materials produced by using the pentaploid as male parent in the backcross with Stamf and no self fertile BC_2S_1 adult plant was obtained. The 311 self-fertile *G. hirsutum* x *G. australe* BC_2 plants were distributed in 18 distinct phenotypic classes. All the plants grouped in a class presented similar qualitative morphological traits (color and shape of the leaves, color of the flowers, relative position of the stigma and the staminal column, size and shape of the capsules). Most of the classes (17 out of 18) came from seeds produced using the pentaploids as male parent and one class came from the backcross to *G. hirsutum* of the pentaploid *G. hirsutum* x *G. australe* used as female parent. Among these 18 phenotypic classes, the one that presented by far the highest number of individuals (249 plants out of 311) showed a very high level of self-fertility (98% of the control seed production) and qualitative traits similar to those of *G. hirsutum* (data not shown). Cytogenetic analysis carried out in Belgium on the progeny of this class confirmed the euploid nature of these plants (2n=4x=52 chromosomes). The frequency of appearance and the fertility of the 17 other phenotypic types were variable. The phenotypic segregation observed in the progeny of thirteen of the eighteen BC_2 phenotypic classes was coherent with the distribution that is expected to be obtained from monosomic addition stocks. Indeed, in the progeny of these 13 phenotypic classes, three types of individuals were found almost systematically: (i) materials that were phenotypically similar to their mother plant (i.e., putative 4x + 1 monosomic addition plants with 53 chromosomes), (ii) individuals with a very restricted level of fertility (sterile or producing less than five seeds per plant) showing an accentuation of some of the mother plant traits (i.e. putative 4x + 2 disomic addition plants, with 54 chromosomes), and (iii) individuals totally similar to *G. hirsutum* (i.e. putative 4x euploid plants, with 52 chromosomes). The cytological and *in ~~situ~~ ~~hybridizations~~situ* hybridization observations carried out in Europe on the progeny of these materials confirmed the presence of one additional alien chromosome in all the putative monosomic addition stocks (Figure 1 and 2). Among the 86 SSRs we used to confirm the origin of the supernumerary chromosome of the thirteen monosomic addition lines 32 SSRs revealed the presence of a *G. australe* specific allo-allele in at least one monosomic addition line (Figure 3). The presence of these *G. australe* specific SSRs in the monosomic addition lines and the fact that these markers were mapped and assigned to chromosomes or homeologous chromosome pairs of the tetraploid genome led us to infer specific chromosomic assignments for each of the monosomic addition lines. Among the 13 monosomic addition stocks we isolated in the BC_2 progeny of *G. hirsutum* x *G. australe* hexaploid, homeologies were found

with eight distinct linkage group pairs of the tetraploid genetic map. This means that some monosomic addition stocks that were considered as different upon the basis of their phenotypic aspect carried actually the same *G. australe* chromosome while three of the MA stocks were introgressed by two fragments of distinct *G. australe* chromosomes. On the basis of the SSR data, seven different MA stocks were identified. The homologies existing between the supernumerary chromosomes of these MA stocks and the chromosome pairs of the tetraploid genome are presented in Table 1. Upon the basis of the existing homologies between the MA stocks established thanks to SSR markers, the frequency of appearance of each of the MA stocks among the population of 311 self-fertile BC_2 plants analyzed in Benin in 1999 was calculated. These data are presented in Table 2 with an assessment of the pollen fertility and of the average seed productivity of each MA stock. Table 3 presents the phenotypic segregation observed in the progeny of the seven MA families while Table 4 contains the main distinctive morphological traits of each monosomic addition line. G_2-A MA plants had a bushy growth habit with short internodes and small light-green tri- to pentalobate leaves. Despite the production of a large number of flower buds, the final number of capsules per plant was ~~low,~~low; they were small with three carpels and a reduced number of viable seeds (generally only one or two). The disomic addition plants presented an accentuation of the MA parent traits and were totally sterile. G_2-B MA plants had a slender growth habit with long vegetative branches, a high density of hairs on dark-green medium sized generally tri-lobate leaves. They produced small capsules surrounded by narrow bracts. The disomic addition plants presented an accentuation of the MA parent traits and had a very reduced fertility (one or two viable seeds per plant). G_2-C MA plants had a pyramidal to slender growth habit and produced slightly embossed medium-sized tri- and pentalobate leaves. Their capsules were big compared to the other MA stocks with 4 to 5 carpels. These plants produced brown lint. The disomic addition plants presented an accentuation of the MA parent traits and were totally sterile. G_2-D MA plants had a pyramidal to slender growth habit. Their leaves were pentalobate with light anthocyanin spots on the petiole. They produced medium sized brevistyle flowers with light-pink petals and anthers and medium-size capsules similar to *G. hirsutum* ones. About 25 % of the seeds produced by these plantS showed a 40 to 50 % reduction of their gossypol content. The disomic addition plants presented an accentuation of the MA parent traits and were totally sterile. G_2-E MA plants had a slender growth habit with a few short vegetative branches. The disomic addition plants were stunted with trilobate leaves and a very low level of fertility (only two viable seeds were produced). G_2-F MA plants had a cluster growth habit with a few very short internodes on the fruiting branches. They produced very small highly cut trilobate leaves and medium-sized flowers similar to the ones of *G. hirsutum*. Their medium-sized capsules had a recurved pedicel. The disomic addition plants had a more slender growth habit, with trilobate light-green leaves, small flowers and indehiscent anthers. No capsule was harvested on these disomic materials. G_2-G MA plants had a pyramidal to slender growth habit with long vegetative branches. Their leaves were slightly embossed. They produced a lot of flowers similar in shape and in size to the ones of *G. hirsutum*. The capsules were small and spherical. Disomic addition plants had a bushy and vegetative growth habit with small leaves. They produced many flowers by only three capsules were harvested with one seed in each.

Discussion and Conclusions

Provided a sufficiently large number of backcrosses are carried out, the hexaploid *G. hirsutum* x *G. australe* constitutes a valuable material to produce BC_1 pentaploids. The use of these pentaploids as male parents in backcrosses to *G. hirsutum* permitted the rather easy isolation of a large number of BC_2 MA stocks compared to the important efforts, including the application of embryo rescue on a large scale, spread by Altman et al (1987) to obtain backcross derivatives and MA plants from the *G. hirsutum* x *G. sturtianum* hexaploid. The same type of results was obtained by Koto (1983) who succeeded to isolate more than ten different MA stocks in the progeny of the *G. hirsutum* x *G. longicalyx* pentaploid when backcrossing the latter as male parent to *G. hirsutum*. On the contrary, the use of the *G. hirsutum* x *G. australe* pentaploids as female parent in backcrosses with Stamf or when self-pollinated gave almost systematically rise to autosterile plants. The same observation was made by Poisson (1970), André and Verschraege (1984), Koto (1983), Altman *et al.* (1987), and Brubaker *et al.* (1999) with bi-specific pentaploid hybrids involving *G. hirsutum* and B, C, E, G, or F genome diploid species.

The autosterile plants obtained in the backcrossed progeny of these pentaploids generally carried several alien chromosomes. This observation also made by Hau (1981), Koto (1983), Poisson (1970), and Schwendiman (1978) indirectly confirms the better tolerance of female gametes to multiple alien chromosome addition in their ~~nucelus~~nucleus and put in evidence the better competitiveness of cotton male gametes carrying only one additional alien chromosome compared to pollen grains carrying several alien chromosomes. Morphologic characters allowing to distinguish without ambiguity the different MA stocks were rather scarce (~~colour~~color of the petals, fibre ~~colour~~color, shape of the pedicel) and often a high level of segregation was observed between the plants carrying the same supernumerary chromosome of *G. australe* for common qualitative and quantitative traits due to the involvement of two different *G. hirsutum* cultivars in the creation of our MA materials. Regarding this point, the SSR markers were very useful to confirm the chromosomic status of the different monosomic addition lines isolated in the backcross progeny of the pentaploid. They revealed genomic homeologies between *G. australe* (genome G_2) chromosomes with those of *G. hirsutum* (genome A_hD_h) thanks to the SSR flanking sequences conserved in the two species and showed that only seven *G. australe* chromosomes were added in single copies to the *G. hirsutum* genome among the 13 monosomic addition families we isolated in the BC_2 progenies of *G. hirsutum* x *G. australe* hexaploids. Three traits of agronomic interest were identified among our MA stocks. The production of capsules with recurved pedicel was observed in the MA plants carrying the chromosome G_2-F. This feature that exists in the nature only in some wild diploid Australian species belonging to K-genome (Craven *et al., 1*994) may assist in reducing capsule rot before dehiscence or rain damage to the lint after dehiscence of the capsule. The diminution of the size of the bracts that surround the reproductive organs was observed in G_2-B monosomic addition stocks. This trait could facilitate the protection of cotton against some of its main pest enemies. A significant, but not drastic reduction of the gossypol content of the seed was observed in the G_2-D monsomic addition materials. This observation confirms the hypothesis made by Benbouza et al (see these proceedings) regarding the polygenic determinism of the “glanded-seed and glandless-plant” trait in the wild diploid Australian cottons carrying it. Contrary to the observation made by Rooney et al (1991) regarding the absence of male transmission of *G. sturtianum* supernumerary chromosomes in the four MA stocks they ~~analysed~~analyzed, disomic addition plants were

produced through natural selfing of our MA stocks. This observation can be linked to the better global fertility of our pentaploid and MA plant compared interspecific hybrid plants involving *G. sturtianum* chromosomes.

In the progeny of the pentaploid and the monosomic addition lines, each alien chromosome addition is ~~characterised~~characterized by a particular transmission rate, which is chromosome specific. Similar observations concerning the variability of the alien additional chromosome transfer rate in the progeny of different pentaploids and monosomic addition materials were made by Poisson (1970), Hau (1981), Koto (1983), André and Verschraege (1984), Rooney *et al.* (1991), and Mergeai *et al.* (1993). These variations can be explained by differences between the alien additional chromosome and its homeolog within the *G. hirsutum* genome. It can also be explained by various factors acting on the viability of the aneuploid male and female gametes, on the aneuploid zygote development, on the aneuploid seed germination, and on the survival of the plants carrying an alien supernumerary chromosome. The disproportionate recovery of G_2-A chromosome suggests that it might undergo preferential transmission in a *G. hirsutum* background as it was observed by Roney & Stelly (1991) for a *G. sturbianum* chromosome in the MA stocks they isolated. Preferential transmission through male or female gametes, or both, has often been noted for monosomic alien addition chromosomes introgressed into a cultivated crop species background (Maan 1975). In most instances, the preferential transmission is caused by a single gene located on the alien chromosome (Maguire 1963). The G_2-A and other preferential transmission systems seem to be potentially useful in plant genetic improvement. These uses include (i) consistent transmission of certain sets of genes, (ii) introgression, (iii) genetic studies, and (iv) specialized use of the genes responsible for preferential transmission. For instance, the manipulation of such system would allow the synthesis of triple cross and double cross hybrids that uniformly contain desired genes from either or both parents. Tsujimoto & Tsunewaki (1984) reported incorporation of an *Aegilops speltoides* gametocidal gene (Gc 1) into *T. aestivum* and proposed linking desirable traits to this gene so that transmission of the desired trait to all progeny would be ensured. Intensive work would be required, but the potential for success is present. If F_2 hybrid cotton cultivars become commercially established, such a mechanism could be used to achieve uniformity among the F_2 plants for key genes, e.G. those engineered into one parent. Globally, between 2 to 3 % of the euploid plants issued from the pentaploid and from our MA stocks showed morphological introgression indices. This rate varied according to the nature of the supernumerary chromosome. Even if we were never able to observe trivalent associations during our cytogenetic analysis which concerned on an average only about twenty pollen mother cells per MA stock, the appearance of these euploid plants with drastic phenotypic changes indicates that intergenomic recombinations and exchange occurred in the MA parent plants or in the earlier generations. The recombination rates we may infer from the morphological evidences we noticed are coherent with the data of Rooney *et al.* (1991) who observed trivalent formation rates varying between 1.3 % and 4.5 % in the four MA stocks carrying supernumerary chromosome of *G. sturtianum* they ~~analysed~~analyzed. In earlier generations, Altman *et al.* (1987), observed an average trivalent rate of 5%. An accurate evaluation of the recombination frequency that occurs in our MA stocks will request additional work involving an extensive use of mapped molecular markers.

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References

Altman, D.W.~~,~~ Stelly, D.M.~~;~~ and Kohel, R.J. (1987). Introgression of the glanded-plant and glandless-seed trait from *Gossypium sturtianum* Willis into cultivated upland cotton using ovule culture. *Crop Science*, **27**: 880-884.

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

André, B.~~,~~ and Verschraege, L.~~,~~ (1984). Amélioration du cotonnier *Gossypium hirsutum* L. par hybridation interspécifique : utilisation de l'espèce *G. areysianum* Delf. Hutch. *Bulletin des Recherches Agronomiques de Gembloux*, **18**~~:~~ 151-164.

Benbouza, H. ~~,~~ Lognay, G. ~~,~~ Palm, R. ~~,~~ Baudoin, J.P. ~~,~~and Mergeai, G. (2002). Development of a visual method to quantify the gossypol content in cotton seeds. *Crop Science*, **42**~~:~~ 1937-1942.

Barrow, J.R. (1981). A new concept in assessing cotton pollen germinability. *Crop Science*, **21**: 441-444.

Brubaker, C.L., Benson C.G. , Miller C., Leach N.D. (1996). Occurence of terpenoid aldehydes and lysigenous cavities in the "glandless" seeds of australian *Gossypium* species. *Australian Journal of Botany*, **44**: 601-612.

Brubaker C.L., Brown, A.H.D.~~,~~ ~~J.McD~~. Stewart, ~~M.J~~ J.McD. Kilby, M.J. and Grace, J.P. ~~Grace.~~ (1999). Production of fertile hybrid germplasm with diploid Australian *Gossypium* species for cotton improvement. *Euphytica*, **108**~~:~~ 199-213.

Craven, L.A.~~,~~ Stewart, J.McD~~,~~ Brown, A.H.D.~~;~~ and Grace, J.P. (1994). The Australian wild species of *Gossypium*. ~~In : Challenging the future, Proceedings of the World cotton research Conference, 1 : Brisbane, Australia, February 14-17, 1994.~~ G. ~~A. Constable and N.W. Forrester (Eds), CSIRO, Melbourne, pp.~~ 278-281.

Demol, J., Verschraege, L.~~ Verschraege,~~ and Marechal, R.~~ Marechal.~~ (1978). Utilisation des espèces sauvages en amélioration cotonnière. Observations sur les caractéristiques technologiques des nouvelles formes allohexaploïdes. *Coton et Fibres Tropicales* ~~:~~ **33**: 327 - 333.

D'hont, A.~~,~~ Grivet, L.~~,~~ Feldmann, P. ~~,~~ Rao, S. ~~,~~ Berding, N. ~~,~~ Glaszmann, J.C. (1996). Characterisation of the double genome structure of modern sugar cane cultivars (*Saccharum* spp.) by molecular cytogenetics. *Mol. Gen. Gent.*, **250**: 405-413.

Dilday, R.H. (1986). Development of cotton plant with glandless seeds and glanded foliage and fruiting forms. *Crop Sci.*, **26**: 639-641.

Hau, B. (1981). Lignées d'addition sur l'espèce *G. hirsutum*. II. Description phénotypique de quelques lignées d'addition monosomique. *Coton Fibres Trop.*, **36**: 285-296.

Koto, E. (1983). Tentative d'utilisation de l'espèce sauvage diploïde Gossypium longicalyx pour l'amélioration de l'espèce cultivée tétraploïde *G. hirsutum* L. par la méthode des lignées d'addition et de substitution. Ph.D. thesis. University of Orsay, France.

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Mis en forme : Français (France)

Lacape, J.-M., Nguyen ,T.-B. ~~Nguyen~~, Thibivilliers, S., ~~Thibivilliers,~~ Bojinov, B., ~~Bojinov,~~ Matz, E.C., ~~Matz,~~ Blewitt, M., ~~Blewitt,~~ Courtois, B. ~~Courtois~~, Cantrell, R.G. , ~~Cantrell,~~ Burr, ~~B. Burr, and~~ B. and Hau, B. (2003). First combined RFLP-SSR-AFLP map of tetraploid cotton based on a *Gossypium hirsutum* x *Gossypium barbadense* backcross population. *Genome*. Accepted.

Maan, S.S. (1975). Exclusive preferential transmission of an alien chromosome in wheat. *Crop Science*, **15**: 287-292.

Maguire, MP. (1963). High transmission frequency of a *Tripsacum* chromosome in corn. *Genetics*, **48**: 1185-1194.

Maréchal, R. (1983). A collection of interspecific hybrids of genus *Gossypium*. *Coton Fibres Trop*., ~~38~~: 240-246.

Mergeai, G. , Noël, J.M., ~~Noël,~~ Louwagie, J. ~~Louwagie,~~ and Baudoin, J.P. ~~Baudoin~~. (1993). Utilisation du cotonnier sauvage *G. areysianum* pour l'amélioration de l'espèce cultivée *G. hirsutum* L. Description de deux nouvelles lignées d'addition monosomiques. *Coton Fibre Trop*., ~~48~~: 231-250.

Mergeai, G. (2003). Forty years of genetic improvement of cotton through interspecific hybridisation at Gembloux Agricultural University : achievement and prospects. ~~World Cotton Research Conference 3.~~These proceedings.

Ndungo, V., ~~;~~ Demol, J., ~~;~~ and Maréchal, R (1988). L'amélioration du cotonnier *Gossypium hirsutum* L. par hybridation interspécifique. Publication agricole n°23, Administration générale de la coopération au Développement, Brussels, Belgium, 92 p.

Poisson, C. (1970). Contribution à l'étude de l'hybridation interspécfique dans le genre *Gossypium* : transfert de matériel génétique de l'espèce sauvage diploïde *G. anomalum* à l'espèce cultivée *G. hirsutum*. Ph.D. thesis. University of Orsay, France.

Risterucci, A.M., Grivet, L., ~~Grivet,~~ N'Goran, J.A.K., ~~N'Goran~~ Pieretti, ~~Pieretti,~~ I., Flament, M.H. ~~Flament,~~ and Linaud, C. ~~Lanaud.~~ (2000). A high density linkage map of *Theobroma cacao* L. *Theor. Appl. Genet*., ~~101~~: 948-955.

Rooney, W.L., Stelly, D.M. ~~Stelly,~~ and Altman, D.W. ~~Altman.~~ (1991). Identification of *four Gossypium sturtianum* monosomic alien addition derivatives from a backcrossing program with *G. hirsutum*. *Crop Science*, ~~;~~ **31**:337-341.

Rooney, W.L. and ~~;~~ Stelly, D.M. (1991). Preferential transmission and somatic elimination of a *Gossypium sturtianum* chromosome in *G. hirsutum*. *Journal of Heredity*, **82**: 151-155.

Schwendiman, J. (1978). L'amélioration du cotonnier *Gossypium hirsutum* par hybridation interspécifique: utilisation des espèces *G. barbadense* et *G. stocksii*. Ph.D. thesis. University of Orsay, France.

Tsujimoto, H. and ~~;~~ Tsunewaki, K. (1984). Gametocidal genes in wheat and its relatives: I. Genetic analyses in common wheat of a gametocidal gene derived from *Aegilops speltoides*. *Canadian Journal of Genetics and Cyology*, **27**: 178-185.

Vroh ~~Bb~~i, I., Harvengt, L., Chandelier, A., Mergeai, G. ~~,~~ and ~~D~~ du Jardin, P. (1996). Improved RAPD amplification of recalcitrant plant DNA by the use of activated charcoal during DNA extraction. *Plant Breeding*, **115**: 205-206.

Mis en forme

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Table 1. Homeologies existing between the supernumerary chrosomomes of *G. australe* in the MA stocks issued from *G. hirsutum* x *G. australe* hybrid and the pairs of *G. hirsutum* chromosomes

MA Stocks	Homeologous *G. hirsutum* chromosome pairs.
G_2-A	c10 – c20
G_2-B	c09 – c23
G_2-C	c06 – c25
G_2-D	c07 – c16
G_2-E	c12 - c26
G_2-F	c03 – c17
G_2-G[†]	A01 – c18

[†] This MA stock was also introgressed by a very short portion of the homeolog of chromosomes c05sup – D04

Table 2. Frequency of appearance, seed productivity and pollen fertility of the BC_2 fertile plants analyzed in Benin

Phenotypes	Frequency (%) of appearance among the self fertile BC_2 materials	No. of seeds plant^{-1} (% Seed no *G. hirsutum* cv. Stamf)	Stainable pollen grains (%)*
G_2-A	22(7.07)	102(41)	96
G_2-B	9(2.89)	200(80)	90
G_2-C	14(4.50)	196(78)	90
G_2-D	5(1.61)	55(22)	94
G_2-E	3(0.96)	210(84)	83
G_2-F	1(0.32)	213(85)	91
G_2-G	1(0.32)	220(88)	86
Other	7(2.25)	From 55(22) to 214(85)	From 83 to 95
G. hirsutum-like	249(80.06)	245(98)	97
Total	311(100.00)	---	---

* Assessed on two flowers on more than 1000 pollen grains

Table 3. Phenotype frequency distribution in the selfed progeny of the seven monosomic addition stocks issued from *G. hirsutum* x *G. australe* hybrid

Phenotypes	G2-A	G2-B	G2-C	G2-D	G2-E	G2-F	G2-G	Total
~~No.~~ (%) Plants grown	10(100)	63(100)	50(100)	34(100)	10(100)	33(100)	16(100)	216(100)
~~No.~~ (%) Plants with *G. hirsutum* phenotype	0(0)	46(73)	39(78)	22(65)	7(70)	18(55)	12(75)	144(67)
~~No.~~ (%) Plants with MA stocks phenotype	8(80)	11(17)	7(14)	8(24)	1(10)	11(33)	2(13)	48(22)
~~No.~~ (%) Plants with disomic addition phen.	2(20)	6(10)	2(4)	2(6)	2(20)	2(6)	1(6)	17(8)
~~No.~~ (%) Plants showing another phenotype	0(0)	0(0)	2(4)	2(6)	0(0)	2(6)	1(6)	7(3)

Table 4. Influence of each of the seven supernumerary chromosome of *G. australe* on *G. hirsutum* phenotype

Chromosome	G_2A	G_2B	G_2C	G_2D	G_2E	G_2F	G_2G
Growth habit	Bushy	Slender	Pyramidal to slender	Pyramidal to slender	Slender	Cluster	Pyramidal to slender
Leaves	Trilobate, medium sized	Tri to penta lobate. Highly pilose	Similar to *G. hirsutum* but slightly embossed	Tri- to penta-lobate with light anthocyanin spots on the petiole	Similar to *G. hirsutum*	Similar to *G. hirsutum*	Medium-sized and slightly embossed
Flower	Brevistyle White petals	White petals with narrow bracts	Longistyle White petals	Brevistyle Light pink petals and anthers	Similar to *G. hirsutum*	Similar to *G. hirsutum*	Longistyle White petals
Capsules	Small sized and scarce	Small sized	Normal sized	Normal sized	Big and elongated	Normal sized with recurved pedicel	Small sized and spherical
Fibre color	White	White	Brown	White	White	White	White