1695 Preferential transmission of *Gossypium sturtianum* chromosome fragments in the progeny of [(*G. hirsutum* x *G. raimondii*)$_2$ x *G. sturtianum*] trispecific hybrid

Dr. Halima Benbouza , FUSAGx, Gembloux, Belgium
Ms. Fatimata Bintou Hassedine Diouf , ENSA, Thiès, Senegal
Dr. Jean Pierre Baudoin , FUSAGx, Gembloux, Belgium
Dr. Guy Mergeai , FUSAGx, Gembloux, Belgium

ABBREVIATION:

HRS: [(*G. hirsutum* x *G. raimondii*)$_2$ x *G. sturtianum*], S (selfing), BC (backcross) SSR (Simple Sequence Repeats), EST (Expressed Sequence Tags), PCR (Polymerase Chain Reaction); GISH (Genomic *In-Situ* Hybridization), MACALs (Multiple Alien Chromosome Addition Lines).

ABSTRACT

The main objective of this study was to verify the existence of preferential transmission mechanisms of alien chromosome segments introgressed from the wild species *G. sturtianum* in selected advanced generations of the trispecies hybrid [(*G. hirsutum* x *G. raimondii*)$_2$ x *G. sturtianum*] (HRS). Crosses using a $S_1/BC_1/BC_2S_2$ plant as male and female parents were carried out with *G. hirsutum* cv STAM F. Two hundred and fourteen mapped SSR markers evenly distributed on the 26 chromosomes of *G. hirsutum* L. were used to monitor the introgression and conservation of SSR loci (alleles) coming from the Australian wild species *G. sturtianum* in the selected progenies. Ten *G. sturtianum*-specific SSR alleles mapped on c2-c14, c3-c17, and c6-c25 linkage groups were conserved in the selfed progenies of the $S_1/BC_1/BC_2S_2$ genotype. A high level of heterozygosity, varying from 49% to 100%, was observed for all conserved *G. sturtianum* SSR markers. Results showed that three SSR markers mapped on c6-c25 linkages groups were systematically transmitted in all selected progenies of the HRS trispecies hybrid. SSR markers mapped on c2 and c3 seemed to be preferentially transmitted via female gametes. The data do not exclude the possibility of the presence of lethality factor(s) on the conserved alien fragments which are expressed in homozygote state and/or post zygotic lethality due to genetic interaction of *G. sturtianum* recessives alleles with *G. hirsutum* genetic background. Possible mechanisms for preferential transmission of alien chromosomes segments are discussed.

Keywords: Microsatellites, *Gossypium*, introgression, preferential transmission, hybrid.

Introduction

Gossypium contains about 50 diploid and tetraploid species distributed worldwide in both tropical and subtropical areas. The tetraploid species (2n=4x=52, AADD) contains two distinct subgenomes which are related to the A genome of the Asiatic cultivated diploid species and D genome of the American wild diploid species (Wendel and Cronn, 2003). Four *Gossypium* species namely *G. arboreum*, *G. barbadense*, *G. herbaceum* and *G. hirsutum* are cultivated, the latter (upland cotton) being by far the most important. The diploid species (2n=2x=26) fall into eight different cytotypes designated A, B, C, D, E, F, G, and K (Endrizi et al., 1985; Stewart, 1995). Genome classifications correlate with the fertility and

frequency of chromosome recombination in interspecific hybrids, and in general, interspecific hybrids within genomes are fertile, recombining readily, whereas intergenomic hybrids are infertile and exhibit limited bivalence during meiosis (Stewart, 1995).

In hybrids, genetic recombination is usually restricted to homoeologous chromosomes. Successful introgression using bridging species occurs when homoeologous recombination is frequent enough that the target genomic region has been introgressed before the donor chromosome is lost during the recurrent backcrossing process to the recipient genome. However, the transfer of desired genes or gene clusters from alien species to superior cultivars is often accompanied by unacceptable wild traits due to inhibitory genes also present in the transferred chromosome segment. Procedures for chromosome pairing manipulation in polyploid crop plants, generally referred to as 'chromosome engineering,' leads to fruitful recombination of entire genomes, parts of genomes or chromosomes segments. The results of such manipulations are genomic reconstruction and led to a reduction of the size of the alien chromosome segment transferred to a crop plant genome (Prem, 2006; Qi et al., 2007).

Determining the frequency at which donor chromosomes are transmitted and whether they have been transmitted intact requires chromosomes-specific markers (Burow et al., 2001). Microsatellite markers in cotton are chromosome-specific and evenly distributed along chromosomes (Liu et al., 2000; Nguyen et al., 2004). Such markers reveal a higher level of polymorphism than RFLP markers.

Preferential loss/recovery of a specific allele, chromosome, or genome between two generations can result from numerous phenomena, e.g., zygotic lethality (Lee, 1981), somatic elimination (Kasha, 1974), or preferential transmission that alters and renders non-random the transmission of the genetic material from meiocytes to zygotes. Lethality of gametes and/or zygotes can be caused by presence of lethal gene (s) introgressed from alien species and their expression depends on the background in which they act. Thus, differential viability of spores, gametes, or zygotes provides distorted transmission frequencies.

Mechanisms that cause preferential transmission are diverse. Loss of univalents during meiosis and fertilization is a simple example of preferential transmission (Rooney and Stelly, 1991). Sandler and Novitski (1957) indicated that heterozygotes of certain constitution fail to produce gametes with equal frequencies as a consequence of meiotic division mechanics. These unequal frequencies affect genes frequencies in populations and such meiotic behaviour was referred as meiotic drive.

The case of meiotic drive in plants causing a preferential transmission was demonstrated from the work of Cameron and Moav (1957). They reported a gene (*Kl*) in *Nicotiana plumbaginifolia* which seems to cause degeneration of pollen not carrying it when the chromosome on which it occurs is added to the *Nicotiana tabacum* complement. Performance of this hybrid and segregation in its progeny reveal that part of pollen is aborted and that nearly all the functional pollen transmits the extra chromosome. Gametocidal (*Gc*) factors in wheat are strong distorters that affect plant fertility through differential functioning of the gametes (Nasuda et al., 1998). They are introduced into wheat trough interspecific hybridisation and backcrossing with related *Aegilops* species and only gametes with the alien chromosome carrying *Gc* factor are functional and the *Gc* factor is selectively transmitted to the progeny (Mann, 1975). Analogous of this case in plants, is the segregation distorter (*SD*) gene of *Drosophila* (Sandler and Hiraizumi, 1960) that somehow brings about the destruction of many of the gametes from heterozygous males

which carry its normal allele. It has been suggested that *SD* induces breakages of its homologue and that gametes which receive the breakage products are deficient and therefore unviable (Sandler and Hiraizumi, 1960).

The work of Rick (1966) revealed that the abortion of male and female gametes in tomato (*Lycopersicom* sp.) is determined by allelic interaction. Three alleles of gamete eliminator gene *Ge* were found, but elimination occurring only in Ge^c/Ge^p, in which Ge^c gametes are aborted. No abortion occurs in combination with Ge^n or in Ge^c/Ge^c or Ge^p/Ge^p. A similar genetic model exists for the pollen killer in wheat (Loegering and Sears, 1963).

In the present paper we report detailed molecular observations of the preferential transmission mechanism of some alien chromosome fragments introgressed from the wild species *G. sturtianum* in selected advanced generations of the trispecies hybrid [(*G. hirsutum* x *G. raimondii*)² x *G. sturtianum*], designated HRS. Microsatellite markers were used to monitor the introgression of DNA fragments coming from the Australian species *G. sturtianum* Willis in a population of derivatives obtained from the HRS trispecies hybrid.

The possible presence and transmission of eliminator allele(s) and/or lethal allele(s) via female or/and male gametes was tested by using the same $S_1/BC_1/BC_2S_2$ plant as male and female parent in crosses with *G. hirsutum* cv STAM F.

MATERIALS AND METHODS

Plant materials and DNA extraction

The plant materials used in this study were obtained in the framework of a program aimed at improving the nutritional quality of the seed by trying to develop upland cotton cultivars having the *glanded-plant* and *glandless-see*d trait of *G. sturtianum* Willis.

Two cultivars of *G. hirsutum* L. $2(A_hD_h)_1$ (NC8 and C2), one accession of *G. raimondii* Ulbr. ($2D_5$), and one accession of *G. sturtianum* Willis ($2C_1$) were used for the creation of the trispecies hybrid HRS (*G. hirsutum* x *G. raimondii* x *G. sturtianum*, $[A_hD_hD_5C_1]$) according to the pseudophyletic introgression method (Mergeai, 2004). The scheme to create the trispecies hybrid is detailed in Vroh Bi et al., (1998). The selected plants were euploid (2n=4x=52) and showed high frequency of chromosome pairing and chiasmata (Mergeai et al., 1997; Vroh Bi et al., 1999a).

One BC_2S_1 plant issued from the HRS hybrid produced seeds with very different levels of gossypol glands and was chosen for its ability to give segregating progenies for this trait (Mergeai et al., 2000). The BC_2S_1 plant was selfed and backcrossed to *G. hirsutum* cultivar STAM F to produce BC_2S_2, BC_1/BC_2S_2, $S_1/BC_1/BC_2S_2$ and $S_2/BC_1/BC_2S_2$ materials (Benbouza et al., 2004). Figure 1 shows the scheme followed to obtain the analysed materials. Only plants resulting from seeds having the lowest level of gossypol glands visible on their kernel wall were retained in each generation. A $S_1/BC_1/BC_2S_2$ genotype exhibiting the *low-gossypol seed* and *high-gossypol plant* trait was auto-pollinated and crossed as male and female parent to *G. hirsutum* cv. STAM F in order to quantify the transmission of the *G. sturtianum* SSR markers conserved in this plant. Table 1 gives the number of plants issued from these pollinations that were screened using mapped SSR markers.

Total genomic DNA was extracted from fresh young leaves. Leave samples of the trispecies hybrid HRS and all its progenies were frozen and ground. Genomic DNA was then isolated

using the method described by Benbouza et al., (2006). DNA was also extracted from $S_2/BC_1/BC_2S_2$ deformed seeds to increase the number of analysed individuals according to the method outlined by Wang et al. (1993). DNA concentration was quantified using a fluoroscan and working stocks (10 ngμl^{-1}) were diluted in H_20 ddw (Merck) and stored at –20C° until PCR amplification.

Microsatellites markers analyses

Mapped simple sequence repeats (SSR) (Nguyen et al., 2004) were used to characterize the trispecies hybrid HRS and its progenies. The method used for SSR analysis is described in Liu et al*.*, (2000). Amplification was performed on MJ Research (Water Town, Mass., USA) PTC 100 and 200 thermal cyclers. After the addition of 20µl of loading buffer (98% formamide, 10 mM EDTA, bromophenol blue, xylene cyanol), the mixes were denatured at 92°C for 3 min, and 5 µl of each sample were loaded onto a 6% polyacrylamide gel with 7.5 M urea and electrophoresed in 0.5% TBE buffer at 110-120 W. A non-radioactive silver staining method was used to reveal amplified SSR products as described in Benbouza et al*.* (2006).

In the selection process of the $S_1/BC_1/BC_2S_2$ parent used in the present study, each of the 26 chromosomes of the cotton genome map was screened with a minimum of four SSR to cover the upland cotton genome (5500 cM). All microsatellites used covered almost the entire length of the chromosomes except for c4 and c16 in which only 108.9 cM out off 189.5 cM and 62.8cM out off 165.8 cM were covered, respectively, with mapped microsatellites. Totally, 215 SSRs were tested on DNA samples including the HRS hybrid [(*G. hirsutum* x *G. raimondii*)² x *G. sturtianum*], *G. sturtianum*, *G. raimondii*, as well as *G. hirsutum* cultivars C2, NC8, STAM F, and TM1 standard, and HRS BC_2, BC_2S_1, BC_2S_2, BC_2S_3, BC_2S_4, BC_2S_5, BC_1/BC_2S_2, $S_1/BC_1/BC_2S_2$ plants. This work led to the identification of 10 *G. sturtianum* SSR markers mapped on c2-c14, c3-c17 and c6-c25 linkage groups that were conserved in a heterozygote state in the selected $S_1/BC_1/BC_2S_2$ parent genotype (Figure 2).

RESULTS AND DISCUSSIONS

Tables 2, 3, and 4 present the results regarding the transfer of the *G. sturtianum* conserved SSR markers in the progenies produced by selfing and backcrossing as male and female parent to *G. hirsutum* cv STAM F, the selected HRS $S_1/BC_1/BC_2S_2$ plant.

SSR markers are PCR-based markers, co-dominant and locus specific (Röder et al., 1998), hence they are an ideal molecular marker for the identification of donor segments linked with traits of interest. In cases where the amplified fragment is present in one parent, only the microsatellite markers that generate the products in the donor parent can be used for genotyping.

For the 10 SSR loci analysed, the "25 % *G. hirsutum* homozygotes, 50 % heterozygotes, and 25% *G. sturtianum* homozygotes" theoretical frequency distribution expected in the progeny obtained by self pollination of a heterozygote was never observed. Most of the 123 $S_2/BC_1/BC_2S_2$ plants were heterozygote for the different SSR markers screened. For these plants, the heterozygosity rate varied from 49% (for BNL3971 mapped on c3-c17) to 100% (for BNL1153 and BNL3359b markers, mapped on c6-c25). Different frequencies of *G. hirsutum* homozygotes were observed at the introgressed SSR loci except for three SSR markers (BNL1153, BNL3436, and BNL3359b) mapped on c6-c25 linkage groups. It must be noted that *G. hirsutum* homozygote frequencies were, respectively, 4%, 15%, and 19% for BNL1153, BNL3436, and BNL3359b markers in HRS BC_2S_5 genotypes (Benbouza, 2004).

These three SSR markers were systematically transmitted to the progeny indicating the possible transfer of these SSR loci through pollen and ovules.

The rates of *G. sturtianum* homozygotes were generally lower than the rates of *G. hirsutum* homozygotes, notably for the markers located on c2-c14, c6-c25 and on the extremities of c3-c17.

None of the *G. sturtianum* SSR markers located on the c2-c14 and c3-c17 linkage groups and only 37.5% of the markers located on c6-c25 linkage group were transmitted to the backcrossed progeny (16 plants) through pollen.

The transmission rate of the *G. sturtianum* SSR loci through the ovule was close to the expected theoretical value of 50 % for the two markers located on c2-c14 linkage group and for more than half of the markers (BNL2443b, BNL226b, BNL3939) located on c3-c17 linkage group. However, it was totally distorted (100 % transfer rate) for the rest of the SSR markers located on c3-c17 and for the three SSR markers located on c6-c25.

The conserved chromosome segments of the wild parent are localized at different sites which should indicate a relatively high level of homoeologous recombination, especially on c3 linkage group if the gene order was preserved during the evolution of *G. sturtianum*. The distance between these conserved markers varied from 8 cM (between BNL3989 and BNL226 mapped on c3) to 64 cM on the map of *G. hirsutum* (between BNL3436 and BNL1153 mapped on c25) (Figure 2) indicating that the introgression of alien chromosome fragments should result from at least two distinct recombination processes for each chromosome if the synteny of the *G. hirsutum* genes is conserved in *G. sturtianum*.

In the first case, homoeologous pairing and recombination can induce a reasonably large chromosome segment introgression. However, in the second case, the introgression of smaller segments can also be the result of chromosomes breakage and repairs during homoeologous interactions.

The number of conserved SSR markers in the selected progeny is different on the c2, c3 and c25 linkage groups (Figure 2), which indicate that introgression of alien chromosome segments, occurred at different scales. The loss of chromosomal segments spanning one or two marker loci has been suggested to be a potential indicator of recombination in intergeneric wheat-barley hybrids (Malysheva et al., 2003). This explains the presence of a small cluster of markers as the remainder of the chromosome is lost. This was also observed by Becerra et al., (2007) in a number of cases on BC_1 and BC_2 multiple alien chromosomes addition lines (MACALs) obtained from (*G. hirsutum* x *G. australe*)² and (*G. hirutum* x *G. sturtianum*)² hexaploids.

The high frequencies of heterozygosity observed for all conserved *G. sturtianum* SSR markers, after several generations of selfing, indicate that the cytogenetic/genetic conditions for obtaining homozygosity at high frequencies are not met. Birhman and Hosaka (2000) outlined self-incompatibility and zygote selection, which cause unequal segregation of alleles. Different factors may act on the viability of the recombined male and female gametes or on zygote development. There are several possible explanations for high transmission of these chromosomes fragments in *G. hirsutum* background. They include (1) presence of gametocidal gene on alien fragments, (2) post zygotic lethality due to genetic interaction of *G. sturtianum* recessives alleles with *G. hirsutum* genetic background, and (3) zygotic lethality due to the presence of a lethality factor (s) on the conserved alien fragments expressed in the homozygote state.

Preferential transmission through male or female gametes, or both, has been noted for monosomic alien addition chromosomes introgressed into 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).

When segregation distorters or *Gc* genes occurs, one of alleles at heterozygous loci transmits to the progeny at higher frequencies than the expected Mendelian ratio (Sandler et al., 1959). During meiosis, alien *Gc* genes, in the hetero- or hemizygous state, induce breakage in chromosomes not carrying the genes. The gametes with the broken chromosomes are deficient for some loci and are often unviable. The viable gametes will be those carrying the gametocidal alien chromosome (Endo, 1979; Nasuda et al., 1998). Rick (1966) has reported gametes eliminator allele (*Ge*) in tomato, which causes abortion of gametes because of allelic interaction. *Ge* allele induces abortion of the gametes carrying the opposite allele, although the homozygote shows no adverse effect on the formation of the gametes.

Our results indicate the presence of the alien SSR markers BNL3436 and BNL1153, mapped on the c6-c25 linkage groups, in all HRS progenies, from the BC_1 to $S_2/BC_1/BC_2S_2$, sampled in our study. We can thus suppose that such gametocide genes may exist on one of the *G. sturtianum* chromosomes fragments introgressed in HRS progeny. Becerra et al., (2007), suppose the possible presence of gametocidal chromosome in *G. australe* species when analysing the frequency of alien chromosome transmission in *Gossypium* hexaploid bridging population. The same gametocidal genes may exist in *G. sturtianum* species.

In cotton, preferential transmission has been observed in many studies. Rooney and Stelly (1991) compared four different monosomic alien addition derivatives (MA), designated C1-A, -B, -C and –D, identified from a series of the *G. sturtianum* MA plants in a *G. hirsutum* CAMD-E background to determine if C1-A is preferentially transmitted through sexual and somatic cell types. The transmission frequencies ranged from 13% to 100% from monosomic parental lines. Ahoton et al., (2004) reported a preferential transmission of the G2-A chromosome of *australe* in monosomic addition lines they isolated on *G. hirsutum*. Vroh Bi et al. (1999b) observed that out of 70 species-specific AFLP loci of the donor parent *G. sturtianum*, four were systematically present in all the backcross progenies of two tri-species hybrids [(*G. hirsutum* x *G. raimondii*)$_2$ x *G. sturtianum*] (HRS) and [(*G. raimondii* x *G. sturtianum*)^2 x *G. hirsutum*] (TSH) suggesting that these fragments were located on chromosomes that were preferentially transmitted.

The absence of the male transmission of *G. sturtianum* supernumerary chromosomes in four MACALs stocks was observed by Becerra et al., (2007). They reported the preferential transmission of the linkage group (Aust-M) in the *G. hirsutum* x *G. australe* multiple alien chromosome addition lines (MACALs). For *G. hirsutum* x *G. sturtianum* (MACALs) Sturt-JQ and Sturt-N linkage groups were present in almost all individuals (91%).

Observations of SSR markers amplification reported in Tables 3 and 4 indicate that the c2 and c3 chromosomes may carry a gene(s) that renders unviable megaspores and microspores that lack the alien gene(s). However, two important facts are observed.

1) CIR228a locus mapped on c3 seems to be associated to a "selective" gene for the viable female and/or male gametes. However, when selfing or using $S_1/BC_1/BC_2S_2$ as male parent, the proportion of *G. hirsutum* homozygote in which the alien fragment is absent varied from 7% to 100%, respectively, (Tables 2, 3). On the contrary, no *G. hirsutum* homozygote was obtained for this locus when using $S_1/BC_1/BC_2S_2$ as female parent (Table 4). It could not be

ascertained whether or not the pollen grains/egg lacking the CIR228a locus had lost its function; they may have been functioning, but just unable to compete in certation for the pollen, or to be fertilized for egg, with those carrying the CIR228a alien locus (allele). Evaluations of pollen fertility, with acetocarmine staining, for $S_2BC_1BC_2S_2$ plants indicated a rather high fertility rate (89%). It must be noted that for this cross the number of analyzed individuals was insufficient to draw a definitive conclusion. Gametophytic unviability associated with absence of a specific allele has been identified in crop species. In wheat (*Triticum aestivum* L.), inheritance of the wheat stem rust resistance gene (*Sr* 11) was distorted due to effects of a linked pollen- killing gene *Ki* on *ki* pollen from *Ki*/*ki* sporophytes (Loegering and Sears, 1963). However in our case, low proportion (7%) of female gametes without the CIR228a fragments were viable and produced plants which were completely *G. hirsutum* homozygote. All these plants carried the c6-c25 alien fragments. Such mechanism was also discovered in *Nicotiana* (Cameron and Moav, 1957).

2) The conserved alien fragments mapped on c2 and c3 seem to be transmitted only through female gametes because all male gametes are unviable when using the selected $S_1/BC_1/BC_2S_2$ genotype as male parent (100% of the progeny are *G. hirsutum* homozygote for the loci mapped on the c2 and c3 chromosomes) and only three SSR markers, out of the 10 SSR conserved, mapped on the c6-c25 linkage where transmitted to 62% of the progeny while 37% of the progeny was *G. hirsutum* homozygote for all the amplified loci (Table 3). However, when selfing the selected $S_1/BC_1/BC_2S_2$ genotype, homozygote of recombined alien fragments were obtained at the loci mapped on the c2 (BNL3590) and c3 (BNL226b, BNL2443 and CIR058) (Table 2). This indicates that there is a proportion of male gametes carrying the c2 and/or c3 alien fragments that survive. Also, when selfing or using the selected $S_1/BC_1/BC_2S_2$ genotype as female parent in backcross with STAMF we observe that only the female gametes carrying the c6-c25 and/or CIR228a survive. Thus, we can suppose that the unviability of zygotes carrying the c2 and c3 alien fragments occurs only when the cells are hemizygous. Sears and Loergering (1961) reported a gene in wheat which causes the early abortion of pollen grains not carrying it, from heterozygous but no hemizygous plants.

One of the possible explanation for these observations is that the recombined chromosomes, c2 and c3, differ in their ability to interact with the host spindle apparatus. Genetic incompatibilities that impair the viability of gametes could also play an important role. The recovery of all SSR markers when using the selected $S_1/BC_1/BC_2S_2$ genotype as female parent in backcross with STAMF or self-pollinated can be considered as a proof of the existing incompatibilities.

The results we obtained regarding the absence of recombinations between BNL3436 and BNL1153 markers mapped on c25 chromosome after several generations of selfing, although these two loci are spanned by 64 cM on the *G. hirsutum* map, raise the question of the conservation of the gene order and spacing in *G. sturtianum*.

In crop species, both inversion and translocation events have been implicated in the genome rearrangements (Livingstone et al., 1999). Brubaker and co-workers (1998), while developing a comparative RFLP map of the allotetraploid cotton and its diploids detected 19 loci order differences among two diploid and two tetraploid genomes. The observed inversions were not fully conservative and two reciprocal translocations were confirmed between four allotetraploid A_t genome chromosomes, as was translocation between the two existent A genome diploids. Similar observations were outlined by Rong et al., (2004) when mapping diploid (D) and tetraploid genome (A_tD_t). They confirmed two reciprocal translocations and several inversions between A_t chromosomes.

For our future investigations, genomic *in-situ* hybridization (GISH) analyses on the selected materials will be used to measure the amount of introgression and to localize the conserved alien fragments. Cytological analyses will permit us to observe and to score chiasmata associations between cytologically marked chromosomes. Further investigation on large populations must be realized to determine the mechanisms of the preferential transmission observed in our study.

REFERENCES

Ahoton, L., J.M. Lacape, A. Dhont, J.P. Baudoin, and G. Mergeai. 2004. Isolation and Characterization of seven alien monosomic addition lines of *Gossypium australe* F. Muell. On *G. hirsutum* L. p. 135-142. *In* Proc. World Cotton Res. Conf-3, Cape Town. South Africa. 9-13 March. 1998. Agricultural Research Council- Institute for Industrial Crop, Pretoria, South Africa.

Becerra, A.L. and C.L. Brubaker. 2007. Frequency and Fidelity of alien chromosome transmission in *Gossypium* hexaploid bridging populations. Genome (submitted).

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

Table 1. Number of plants screened with SSR markers issued from the selfing and the backcrossing to *G. hirsutum* cv STAMF of the selected $S_1/BC_1/BC_2S_2$ plant

Origin of the seeds	Number of screened plants
Auto pollination	123
G. hirsutum cv. STAM F * $S_1/BC_1/BC_2S_2$ ♂	16
$S_1/BC_1/BC_2S_2$ ♀* *G. hirsutum* cv. STAM F	13

Table 2. Quantification of the transfer of SSR markers through auto pollination of the selected $S_1/BC_1/BC_2S_2$.

Chromosome	conserved SSR markers	Homozygote *G. hirsutum* (%)	Heterozygote (%)	Homozygote *G. sturtianum* (%)	Number of individuals without DNA amplification
	BNL3590	15 (13)	101 (86)	1 (0.9)	6
	BNL3971	61 (51)	60 (49)	0 (0)	2
	BNL2443b	9 (8)	101 (89)	4 (4)	9
	BNL226b	10 (9)	95 (82)	10 (9)	8
	BNL3989	11 (13)	110 (91)	0 (0)	2
	CIR058	23 (20,4)	75 (66,4)	15 (13,3)	10
	CIR228a	9 (7)	112 (93)	0 (0)	2
	BNL3359b	0 (0)	119 (100)	0 (0)	4
	BNL3436	0 (0)	119 (99)	1 (0,9)	3
	BNL1153	0 (0)	116 (100)	0 (0)	7

Table 3. Quantification of the transfer of SSR markers through microspores in the backcrossed progeny of the selected $S_1/BC_1/BC_2S_2$.

Chromosomes	conserved SSR markers	Homozygote *G. hirsutum* (%)	Heterozygote (%)	Homozygote *G. sturtianum* (%)
c2	BNL3590	16 (100)	0	0
	BNL3971	16 (100)	0	0
	BNL2443b	16 (100)	0	0
	BNL226b	16 (100)	0	0
	BNL3989	16 (100)	0	0
	CIR058	16 (100)	0	0
	CIR228a	16 (100)	0	0
c6	BNL3359b	10 (62,5)	6 (37,5)	0
c25	BNL3436	10 (62,5)	6 (37,5)	0
	BNL1153	10 (62,5)	6 (37,5)	0

Table 4. Quantification of the transfer of SSR markers through megaspores in the backcrossed progeny of the selected $S_1/BC_1/BC_2S_2$.

Chromosomes	conserved SSR markers	Homozygote *G. hirsutum* (%)	Heterozygote (%)	Homozygote *G. sturtianum* (%)
c2	BNL3590	6 (46,2)	7 (53,9)	0
	BNL3971	8 (51,6)	5 (38,5)	0
c3	BNL2443b	5 (38,5)	8 (51,6)	0
	BNL226b	6 (46,2)	7 (53,9)	0
	BNL3989	7 (53,9)	6 (46,2)	0
	CIR058	0	13 (100)	0
	CIR228a	0	13 (100)	0
c6	BNL3359b	0	13 (100)	0
	BNL3436	0	13 (100)	0
c25	BNL1153	0	13 (100)	0

Figure 1: selection scheme for $S_2BC_1BC_2S_2$(8)3G14 genotypes.

G.h x *G.r* x *G.s*

G.h

X

*

HRS $(AD)_1D_5C_1$ $2(AD)_1$

BC1

G.h

X

*

BC2

G.h

X

*

BC2S1

BC2S2

X

G.h

BC1/BC2S2

S1/BC1/BC2S2

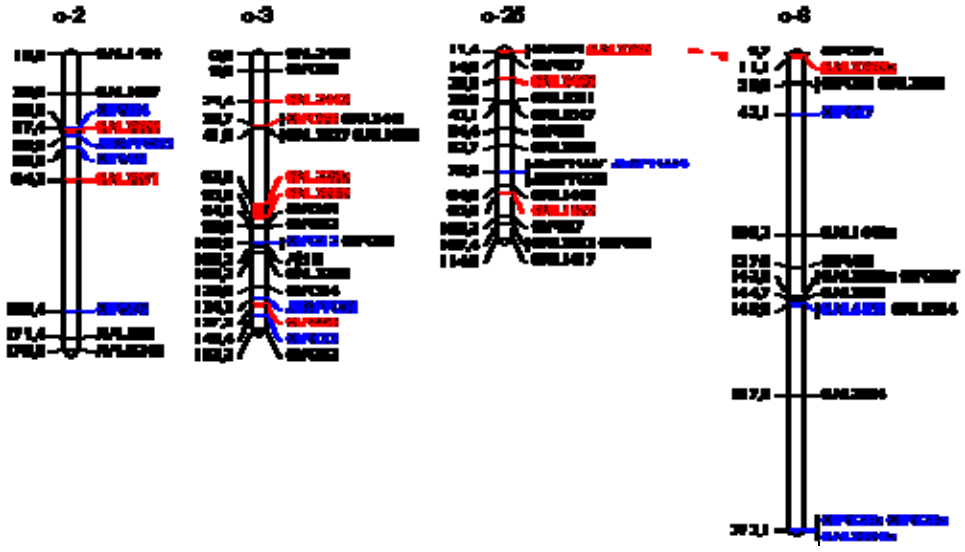
G.h

X

S2/BC1/BC2S2

BC1/S2/BC1/BC2S2

Figure 2. Mapped SSR markers introgressed and conserved from *G. sturtianum* in $S_2/BC_1/BC_2S_2$ genotypes.



Legend:

G. h: *G. hirsutum; G. r*: *G. raimondii*; *G. s: G. sturtianum*; HRS: allotetraploid trispecies hybrid.

Figure 2. Mapped SSR markers introgressed and conserved from *G. sturtianum* in $S_2/BC_1/BC_2S_2$ genotypes.

Legend:

Blue color: non polymorphic SSR; Red color: conserved SSR; Black color: polymorphic SSR.