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Introduction of East African Diploid Cotton Genetic Variation Into Upland Cotton

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Abstract

The African wild diploid cotton species, *Gossypium longicalyx* Hutch. & Lee. (2n = 2x = 26, F_1F_1) presents many valuable traits that can be introduced into *Gossypium hirsutum* to enhance its narrow genetic basis. To assess the possibility of using monosomic alien addition line (MAAL) of *G. longicalyx* in *G. hirsutum* in an interspecific breeding program, the progeny of ten MAALs was characterized. Chromosome counting allowed to identify the addition of single alien chromosome in 9 of the 10 lines studied. The analysis of the chromosome configurations at metaphase showed the presence of multivalent associations involving the supernumerary chromosome of *G. longicalyx*, indicating the occurrence of recombination between the *G. longicalyx* and *G. hirsutum* chromosomes. The use of microsatellite markers provided evidence of multiple introgressions of *G. longicalyx* DNA in the recipient species. It appeared from the SSR analysis that only four different supernumerary alien chromosomes were present in the studied MAALs. These results confirm the low genetic distance existing between the chromosomes of *G. longicalyx* and those of Ah sub-genome. They highlight the opportunities and constraints associated with the use of *G. longicalyx* in a breeding program of upland cotton.

Keywords: cotton, *Gossypium hirsutum*, monosomic alien addition line, *G. longicalyx*

1. Introduction

Upland cotton belongs to the *Malvaceae family* and to genus *Gossypium* which consists of about 5 allotetraploid species (2n = 4x = 52) and more than 45 diploid species (2n = 2x = 26) (Fryxell et al., 1992; Percival et al., 1999; Ulloa et al., 2007). They are distributed in 9 genomic types AD, A, B, C, D, E, F, G, and K (Percival et al., 1999). *Gossypium* species are classified in gene pools depending on the fluency with which genes could be transferred from them to *G. hirsutum*. The primary gene pool contains all the *Gossypium* allotetraploids (2AD). Among these species, crosses are easy and the recombination frequencies are high. The secondary pool consists of A, D, B and F diploid genomes. A, B and F genomes are genetically close to A subgenome of AD cotton while D genome is directly related to D subgenome Once a fertile hybrid is produced, these genomes have a relatively high recombination frequency. The crosses with the tertiary gene pool including C, E, G, K genome *Gossypium* species are difficult with low recombination rate (Mergeai, 2006).

The diversity of the *G. hirsutum* germplasm base is narrow because of its domestication (Brubaker et al., 1999) and intensive selection for yield, early maturity and cultivation adaptation (May, 1999). Species belonging to primary, secondary, and tertiary gene pools constitue interesting sources of diversity. The sole F-genome species, *Gossypium longicalyx* can provide many desirable traits, such as a finer, longer and stronger fiber, with a resistance to drought and immunity to the reniform nematode *Rotylenchulus reniformis* Lind. & Oliveira (Demol et al., 1978; Yik & Birchfield, 1984; Robinson et al., 2005). *G. longicalyx* is geographically close to a point of the A genome area of extension; they both present similar chromosome and genome sizes. Considering the high number of bivalents counted in AD × F allotriploid, recombination is expected to take part with the

chromosomes of the A subgenome more than with those of the D subgenome. The genomes of *G. longicalyx* and *G. hirsutum* may have a relatively high recombination frequency once a fertile hybrid is produced (Stewart, 1995).

Many attempts have been made to complete introgression of the economic traits of *G. longicalyx* into *G. hirsutum* (Phillips & Strickland, 1966; Demol, 1978; Koto, 1983; Frerich, 1995) through the exploitation of monosomic alien addition lines (MAALs) exploitations.

MAALs carry one chromosome of the wild species in the genetic background of *G. hirsutum*. They provide valuable material for gene introgression and study (Peterka et al., 2004; Fang et al., 2004; Becerra Lopez-Lavalle et al., 2007; Fu et al., 2012). Introgressions have been pursued and achieved using MAALs in many crops such as wheat (Kong et al., 2008), rice (Jena et Khush, 1990; Multani et al., 2003), sugar beet (Gao et Jung, 2002) and cucumber (Chen et al., 2004). In the genus *Gossypium*, the development of MAALs has been reported from the following species In the genus *Gossypium*, the development of MAALs has been reported from the following species: *G. stocksii* (Schewdiman, 1978; Hau, 1981); *G. anomalum* (Hau, 1981), *G. longicalyx* (Koto, 1983), *G. sturtianum* (Rooney & Stelly, 1991), *G. areysianum* (Mergeai et al., 1993), *G. sturtianum* (Ahoton et al., 2003), *G. somalense* (Zhou et al., 2004), *G. sturtianum* (Sarr et al., 2011) and *G. anomalum* (Meng et al., 2020)..

The objective of this work is i) to confirm the karyotype of the putative MAALs, and ii) to monitor the introgression of *G. longicalyx* chromosome fragments in the progenies of these stocks using SSR markers.

2. Materials and Methods

2.1 Plant Material

The plant material consisted of the following genotypes:

i) A variety of Congolese origin *G. hirsutum* L.: cultivar C2 (2n = 4x = 52, $A_hA_hD_hD_h$) (G107) and the accession G17 of *G. longicalyx* (2n = 2x = 26, F_lF_l) (G17) both present in the collections of Gembloux Agro Biotech's greenhouses were used for the creation of the allohexaploid (*Gossypium hirsutum* L. × *Gossypium longicalyx*)^2 (G368) by Koto (1983) according to the aphyletic introgression method (Figure 1) (Mergeai, 2003).

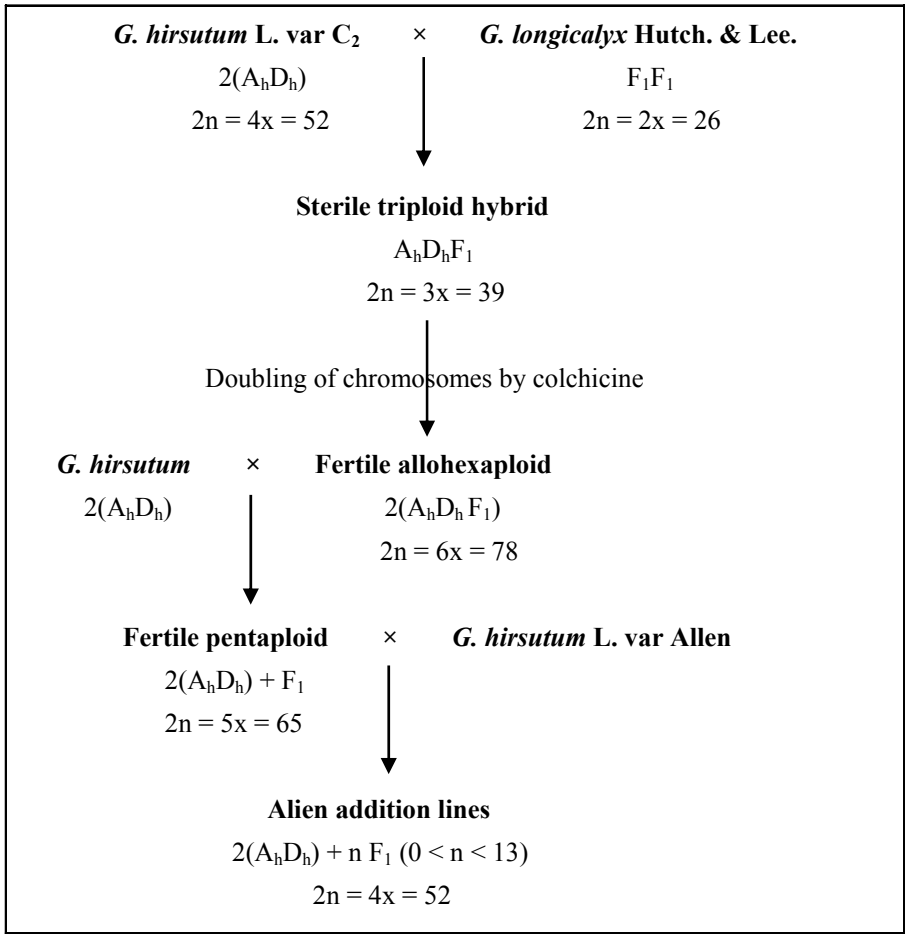


Figure 1. Scheme of the aphyletic method

ii) The selfed progeny of the allohexaploid (*Gossypium hirsutum* L. × *Gossypium longicalyx*)^2 (G368).

iii) The selfed progeny of the 10 MAALs (monosomic alien addition lines), numbered I to XII, obtained by Koto (1983) in the progeny of the bispecific hexaploid (*G. hirsutum* cv. C2 × *G. longicalyx*)^2 (G368) that was created in Gembloux according to the aphyletic introgression scheme (Figure 1) (Mergeai, 2003). This plant material was provided by the gene bank of CIRAD (France).

G numbers correspond to the classification of the accessions and hybrids in the Gembloux Agro-BioTech Cotton Gene Bank (Maréchal, 1983).

The numbering of chromosomes was etablished from to the phenotypic correlation between the isolated types and those of *G. anomalum* and *G.stocksii* MAALs described by Poisson (1970), Schwendiman(1978) and Hau (1981).

The progenies of MAALs (MAAL I to X) were pre-germinated in steam at 30 °C for 48 hours and then grown in pots in the Gembloux Agro-BioTech greenhouses where no effective control of the growing conditions was possible; light, temperature and relative humidity were mostly influenced by outside conditions and were very variable. The relative humidity was 25-45%, and the temperature in the greenhouse varied from 25 °C to 55 °C during the day and from 18 °C to 35 °C at night.

2.2 DNA Extraction

Total genomic DNA was extracted from the young leaves of the two parents, *G. hirsutum* and *G. longicalyx*; the allohexaploid; and the MAAL progeny as described in the protocol of Benbouza et al. (2006a). Total genomic DNA was extracted with chloroform isoamyl alcohol (24:1) and precipitated with isopropanol. Each extracted DNA pellet was suspended in 50 µl TE and incubated overnight at room temperature before being stored at -20 °C.

2.3 SSR Genotyping

The microsatellite (SSR) markers used in this study were developed at Brookhaven National Laboratory (prefix BNL) and at CIRAD (prefix CIR)**.** The SSR markers were tested on the plant material in order to monitor the introgression of *G. longicalyx* chromosome fragments in *G. hirsutum* genetic background Total genomic DNA was extracted from young leaves of the plant material. PCR amplification was performed with PTC 100 and 200 Thermal Cyclers following the protocol by N'guyen et al. (2004).

After the addition of 10 µl of loading buffer (98% formamide, 10 mM EDTA, bromophenol blue, and xylene cyanol), the PCR products were denaturated at 92 °C for 2 min. Then, 5 µl of each sample was loaded onto a 6% polyacrylamide gel with 7.5 M urea and electrophoresed in 1X TBE buffer at 110-120 W. Amplified SSR products were revealed by a silver staining technique (Benbouza et al., 2006b). Each of the thirteen linkage groups was screened with a minimum of five SSRs, except for the chromosome C3-C17, C9-C23, C11-C18 and C13-21, for which 3 SSR were used. Eighty-five pairs of SSR primers reported by N'guyen et al. (2004) were tested on the plant material.

2.4 Cytogenetic Identification

2.4.1 Mitotic Observations

Freshly emerged root tips were used to determine the chromosome number of plants according to the protocol of D'hont et al. (1995). Chromosomes were counted in mitotic cells at metaphase. Young roots were excised and treated in 0.04% hydroxyquinoline at room temperature for 4 hours in the dark. The roots were fixed at metaphase in ethanol/glacial acetic acid (3:1) for 48 hours. Then, the roots were stored in 70% ethanol at 4 °C. After being washed in distilled water, the roots were hydrolyzed in hydrochloric acid and washed in distilled water and citrate buffer. The root tips were subjected to enzymatic maceration in an enzyme (5% cellulose Onozuka R-10, 1% pectolyase Y-23 in citrate buffer) at 37 °C for 1 hour. The tissues were then squashed onto slides in fresh fixative (3:1 ethanol:acetic acid). Chromosome preparations were air dried and stained with 4', 6-diamidino-2-phenylindole (DAPI)/VECTASHIELD before visualization and chromosome counting with a fluorescent light microscope.

2.4.2 Meiotic Observation

Meiotic analysis was performed on the pollen mother cells. Flower buds were selected, fixed in fresh Carnoy's II fluid (glacial acetic acid 1: chloroform 3: and ethanol 6) for 72 hours at 4 °C and then stored in 70% ethanol at 4 °C until analysis. Stamens were lacerated, and anthers were stained with 1.5% acetocarmine solution on a microscope slide. Chromosome staining was enhanced by heating up the sample between slide and coverslip over a flame. Chromosome analyses were performed at metaphase I with a Nikon Eclipse E800 photomicroscope (Nikon, Tokyo, Japan) under oil immersion.

3. Results

3.1 Morphological Traits of the MAALs

From seed setting and seedlings observed with the seed samples received from CIRAD are summarized in Table 1. The average germination rate of the seeds was high (70%), and the survival rate of the germinated seed was 52%. Most of the plants presented the morphological traits of *G. hirsutum* (*i.e.*, putative 4x euploid plants with 52 chromosomes), and the other plants (38.5 %) had a distinctive phenotype (*i.e.*, putative 4x + 1 monosomic addition plants with 53 chromosomes and putative 4x euploid plants carrying introgressed fragments). We noticed that plants of same and different lines presented a heterogenous development, particularly, plants of MAAL F_1 IX have a very slow growth rates (Figure 2). The morphological characters of the MAAL progeny, such as the leaf color, leaf shape, lobule number, shape and size of boll, and flowers, were observed (Table 2).

Table 1. MAALs seed germination and plant development[1]

Putative MAALs	F_1I	F_1II	F_1III	F_1IV	F_1V	F_1VI	F_1VII	F_1VIII	F_1IX	F_1X
No. seeds sown	14	35	32	22	29	10	19	30	25	13
No. germinated seed (%)	10(71)	25(71)	26(81)	11(50)	23(79)	8(75)	11(58)	21(70)	14(56)	10(77)
No. plants grown (%)	4(40)	19(76)	11(42.3)	7(63.7)	19(82.6)	4(50)	2(18.2)	8(38)	2(14.3)	7(70)
No. (%) plants with *G. hirsutum* phenotype	1(25)	21(100)	9(64.28)	5(71.42)	7(33.33)	2(50)	7(70)	2(14.28)	4(80)	4(66.66)
No. (%) 53 chromosomes plants corresponding to the phenotype described by Koto 1983	1(25)	0	4(28.57)	1(14.28)	14(61.9)	2(50)	2(20)	12(71.42)	1(20)	2(33.33)
No. (%) 53 chromosomes plants with another phenotype					1(4.76)					
No. (%) 52 chromosomes introgressed plants	2(50)	0	3(21.42)	1(14.28)	0	0	1(10)	2(14.28)	0	0
No. of plants with a determined karyotype	4	21	16	7	21	4	10	16	5	6

Note. [1] Number of plants followed by the frequency (%).

Table 2. MAALs morphological traits

Genotype	Morphological features
G. hirsutum var Allen	Few vegetative branches, leaves with 3-5 lobes, white flower, rounded 4- to 5-celled boll.
G. hirsutum var C2	A shrub with leaves with 3-5 lobes, round or ovoid 3- to 5-celled boll
G. longicalyx	Crawling shrub, slender stem, pollen color deep yellow, leaves deeply divided triangular lobes, ovoid boll elongated with acute tip, 3 locules, 2 to 3 seeds per locule
Hexaploid	Crawling shrub, dark green small leaves, small ovoid boll with 3 lobes
MAAL F_1 I	Small plant, well-branched, small and dark-green leaves with 3-5 lobes, globular boll
MAAL F_1 II	Glabrous plant, many small leaves with 3-5 acute lobes, rounded three- or four-celled boll
MAAL F_1 III	Slender stem, little branching, large leaves with 4-5 lobes, abundant anthers and pollen, few bolls produced, large amount of cottonseed per boll, ovoid capsule
MAAL F_1 IV	Globular plant, few fruiting branches, small leaves, capsule globular and pointed
MAAL F_1 V	Small plant, small leaves, slow growing, round boll
MAAL F_1 VII	Light green leaves with 3-5 lobes, few fruiting branches, few bolls produced
MAAL F_1 VIII	Small bushy plant, many vegetative branches, light green leaves with 3 lobes, small round boll with 3-4 locules
MAAL F_1 IX	Slow-growing plant, large leaves with 5-7 lobes, low pollen production, short fruiting branches
MAAL F_1 XI	Dark green leaves with 3-5 lobes, many vegetative branches, few small bolls, large number of cottonseeds
MAAL F_1 XII	Slender stem, small leaves with 3 lobes, few fruiting branches, large number of small globular bolls

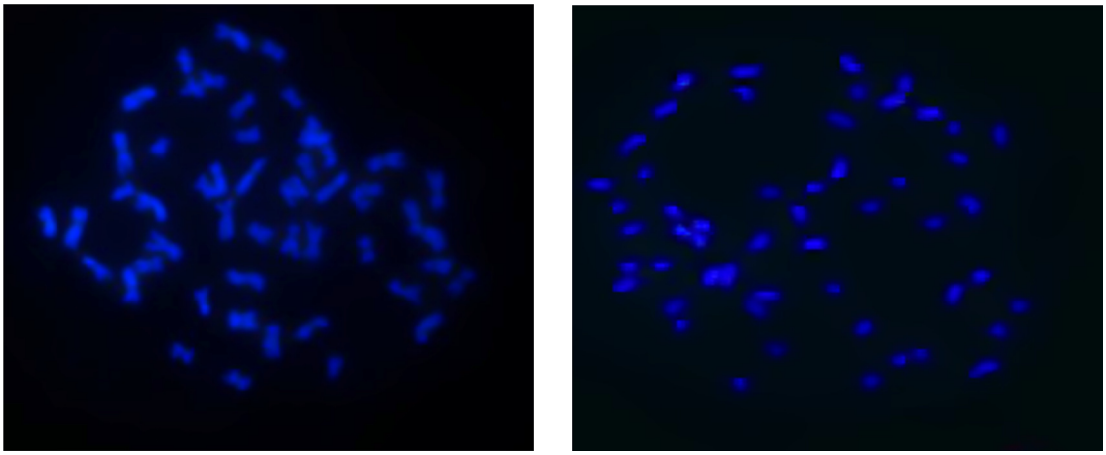
3.2 Cytogenetic Analysis

Classical cytogenetic analysis revealed plants with either 52, 53 or 54 chromosomes per cell (Figure 3). The chromosome number is shown in Table 3. The highest frequency of plants was found with chromosome number 2n = 52. A large number of plants (41.3%) carried a supernumerary chromosome identify (2n = 53) (MAAL F_1 II, MAAL F_1 III, MAAL F_1 IV, MAAL F_1 VIII, MAAL F_1 XI, MAAL F_1 XII). The transmission rates varied widely among the MAALS, MAAL F_1 III showed the highest frequency of plants with 53 chromosomes (71.43%), followed by MAAL F_1 V and MAAL F_1 VIII (66.66%). No MAAL was isolated for MAAL F_1 II. Of the ten lines analyzed, an extra chromosome was found in the mitotic plates of plants belonging to 9 lines (Table 3). In total, the highest frequency of plant was found with chromosome number 2n = 52 (56%), followed by 2n = 53 (33%). Some plants exhibiting a particular phenotype such as a slender steam, large leaves, small bushy plant, small boll was found to carry 2n = 52 chromosomes (10%).

Table 3. Frequency of chromosome number in the MAAL progenies

Line	Number of plants observed	Frequency of plants (%)			
		Chromosome number			
		52	52 with particular phenotype	53	54
MAAL F_1 I	4	25	50	25	
MAAL F_1 II	21	100		0	
MAAL F_1 III	14	64.28	21.42	28.57	7.14
MAAL F_1 IV	7	71.42	14.28	14.28	
MAAL F_1 V	21	33.33		66.66	
MAAL F_1 VII	4	50		50	
MAAL F_1 VIII	10	70	10	20	
MAAL F_1 IX	17	14.28	14.28	71.42	
MAAL F_1 XI	5	80		20	
MAAL F_1 XII	6	66.66		33.33	
Total	109	56.10	10.28	33	0.72

Figure 2. Morphology of the monosomic alien addition lines.
a) MAAL F_1 IX-6; b) MAAL F_1 III-1; c) MAAL F_1 III-10; d) MAAL F_1 VIII-4



a **b**

Figure 3. Chromosomal configuration at the somatic metaphase. a) 52 chromosomes revealed by counterstaining with DAPI (X1000); b) 53 chromosomes revealed by counterstaining with DAPI (× 1000)

We were concerned by the pairing of the chromosomes at metaphase I and how that might affect the recombination rate, which needed to be sufficient for introgression to occur between the alien chromosome and the recipient species *G. hirsutum*. The karyotype of the MAALS (2n = 53) and the euploids (2n = 52 chromosomes) presenting the introgressed phenotype were confirmed by chromosome counts in the PMCs at metaphase I (Figure 4). Meiotic analysis was undertaken on *G. hirsutum* as control. While the division was expected to be synchronized in the anther, we observed in MAAL progenies cells presenting different stages of development in the same anther, revealing that PMC meiosis was asynchronous.

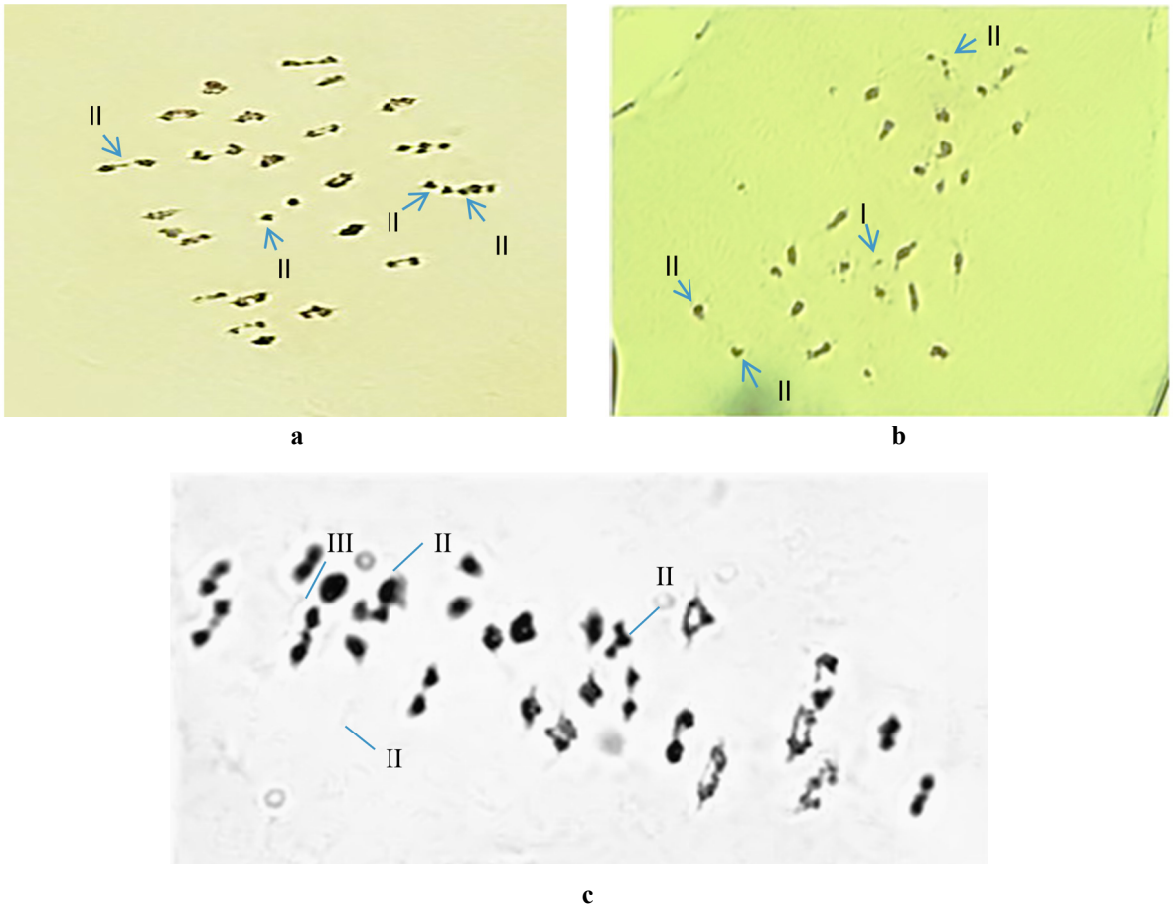


Figure 4. Meiotic metaphase plates: a) *G. hirsutum* (52 chromosomes): 26 bivalents (× 600); b) MAAL F_1 XII-4 (53 chromosomes): 26 II + 1I; c) MAAL F_1 III-1 (53 chromosomes): 25II + 1III

G. hirsutum and euploid plants showed 26 bivalents at metaphase 1 (Figure 4a).

The MAALs presented variable associations with univalents, bivalents and multivalents (Figures 4b and 4c). In most cases, the MAALs presented 26 bivalents and 1 univalent. The trivalent configurations involving 2 chromosomes of *G. hirsutum* and the extra chromosome belonging to *G. longicalyx* were few (Table 4). Most chromosomes presented a loop configuration, but we also observed linear pairing.

Table 4. Mean meiotic chromosome configurations in bispecific hybrids of *Gossypium*

Genomic formulae	Chromosome number	Number of plates	Chromosome configuration						
			I	II	III	IV	V	VI	VII
ADF	39		22.5	6.78	0.93	0.09	0.01	0	**0**
$2(A_hD_hF_1)^a$	78	-	1.47	35.28	0.28	1.22	0.03	0.02	0
$2(A_hD_hF_1)^b$	78	-	2.04	35.75	0.33	0.8	0.03	0.02	**0**
$2(AD)_1 + 1$	**53**	13	0.77	25.62	0.23	0.08	0	0	0
$2(AD)_1$	**52**	23	0.26	25.65	0.09	0.04	0	0	0
$2(AD)_1 + 1$	**53**	8	0.38	25.38	0.63	0	0	0	0
$2(AD)_1 + 1$	**53**	20	0.7	25.6	0.3	0.05	0	0	0
$2(AD)_1 + 1$	**53**	7	0.71	25.71	0.29	0	0	0	0
$2(AD)_1$	**52**	20	0.1	25.85	0	0.05	0	0	0
$2(AD)_1 + 1$	**53**	9	0.78	25.78	0.22	0	0	0	0
$2(AD)_1 + 1$	**53**	22	0.91	25.36	0.45	0	0	0	0
$2(AD)_1 + 1$	**53**	21	0.71	25.62	0.29	0.05	0	0	0

Note. [a] Phillips and Strickland (1966), Schwendiman et al. (1980); [b] Koto (1983).

3.3 Molecular Analysis by SSR Marker

Plants with 2n = 53 and plants with 2n = 52 that presented an introgressed phenotype were used for further characterization.

All primers used in this study were initially used to screen for genetic polymorphism between the parental species *G. longicalyx* (F_1) and *G. hirsutum* (AD_1). We observed that 34 SSRs were monomorphic. The remaining 51 SSRs presented an unequivocal polymorphism (60 % of the primers) between the parents and were used to determine the presence of specific chromosomes of *G. longicalyx* in the MAAL progeny. The plants were analyzed with at least two specific chromosome markers for each of thirteen linkage groups: 31 of the polymorphic SSRs could not be found in the MAALs, 1 was absent in both the hexaploid and the MAALs, 12 showed the presence of *G. longicalyx* alleles in the MAALs and the introgressed euploids, and the remaining were inconclusive (Table 5). An example of SSR electrophoresis profile of the primer BNL4030 showing allele of *G. longicalyx* present in the MAAL progeny is illustrated in Figure 5. None of the *G. longicalyx*-specific SSR markers located on the C2-C14, C3-C17, C5-C19, C8-C24, C9-C23, C11-C21, C12-C26, and C13-C18 were found in the MAALs progeny.

Table 5. Segregation of SSR polymorphic loci in the alien addition lines

Linkage Groups	Polymorphic loci	Alien additions lines									
		MAAL F_1 I	MAAL F_1 II	MAAL F_1 III	MAAL F_1 IV	MAAL F_1 V	MAAL F_1 VII	MAAL F_1 VIII	MAAL F_1 IX	MAAL F_1 XI	MAAL F_1 XII
	BNL1693	-	-	-	-	-	-	-	x	-	-
	CIR009	-	-	-	-	-	-	-	x	-	-
C1-C15	CIR222	x	-	x	X	-	-	-	x	x	x
	BNL530	x	-	x	-	-	-	-	x	x	x
	BNL4030	x	-	x	-	-	x	-	x	x	x
	BNL3594	-	-	x	-	x	-	-	-	-	x
	BNL3103	-	-	x	-	-	-	-	-	-	-
C6-C25	BNL1153	-	x	-	-	-	-	-	-	-	-
	BNL1417	-	-	x	-	-	-	-	-	-	-
	BNL1047	-	-	x	-	-	-	-	-	-	-
C7-C16	CIR141	-	x	-	-	-	-	-	-	-	-
C10-C20	BNL256	-	-	-	-	-	x	X	-	-	-

Note. X: presence of *G. longicalyx*-specific locus marker; -: absence of *G. longicalyx*-specific locus marker.

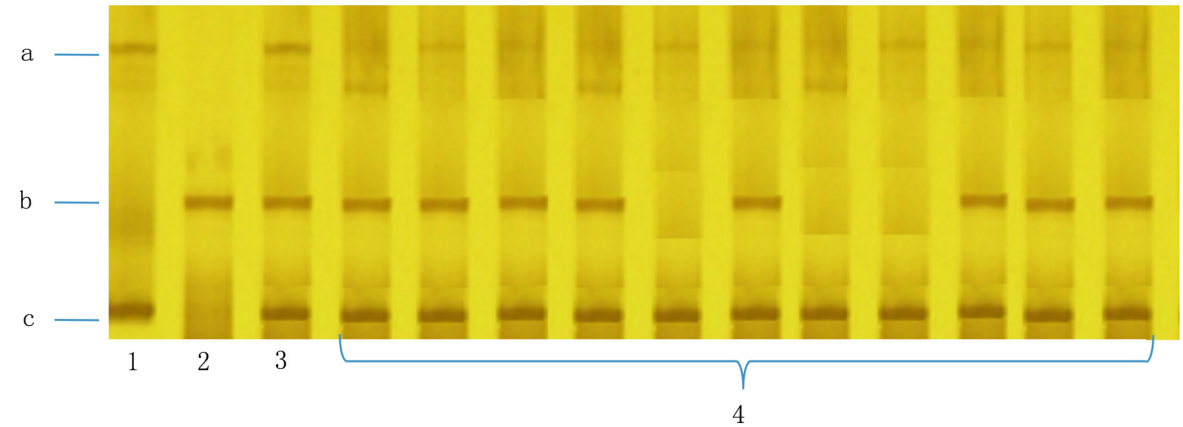


Figure 5. SSR electrophoresis profile of the primer BNL4030 showing allele of *G. longicalyx* present in the MAAL progeny. 1) *G. hirsutum*; 2) *G. longicalyx*; 3) hexaploid (*G. hirsutum* × *G. longicalyx*); and 4) MAAL progeny. a-c: *G. hirsutum*-specific alleles; b: *G. longicalyx*-specific alleles

4. Discussion

The transfer of agronomic traits from wild to cultivated species is laborious due to the genetic distance between the species. Many attempts have been made to isolate monosomic alien addition lines of diploid *Gossypium*

species in *G. hirsutum*. These works used classical cytogenetic analysis combined with morphological observations (Hau, 1981; Koto, 1983; Rooney & Stelly, 1991; Mergeai, 1992). Using news methods, such as molecular genetic markers and molecular cytogenetic techniques, Zhou et al. (2004) isolated two MAALs of *G. somalense* in *G. hirsutum* (Ahoton et al., 2003; Sarr et al., 2011; Chen et al., 2014).

Respectively identified six, five and thirteen of the possible MAALs of *G. australe* chromosomes in *G. hirsutum*, Meng et al. (2020) reported the development of a complete set of 13 MAALs of *G. anomalum* in *G. hirsutum*.

The difficulty in obtaining MAALs is in the triploid hybrid sterility and the production of first generation derivatives from pentaploids. Fertility is restored by colchicine treatment, and MAALs are obtained by repeated backcrossing of the hexaploid to *G. hirsutum*, followed by selection. In this study, Pre-zygotic barriers can explain the low seed set observed in some MAALs.

A large proportion of the plants presenting a distinctive phenotype were found to be MAAL or euploid plants carrying introgressed fragments. The effect of single supernumerary chromosome on phenotype has been reported in cucumber (Chen et al., 2004), allium (Vu et al., 2012) and cotton (Hau, 1981; Koto, 1983; Mergeai, 1992; Ahoton, 2002; Sarr et al., 2012; Chen et al., 2014).

A low transmission of the alien chromosome was observed in all MAALs except for MAAL F_l IX, MAAL F_l V and MAAL F_l VII carrying SSR markers associated respectively to the C1-C15, C4-C22 and C6-C25 linkage groups. The average alien chromosome transmission in this study was 33% (Table 3). The average alien chromosome transmission in the self-progeny of 3 MAALS of *G. sturtianum* in *G. hirsutum* was 23% (Rooney & Stelly, 1991). Two MAALS of *G. areysianum* in *G. hirsutum* presented an alien chromosome transmission of 52% (Mergeai, 1992).

Working on alien addition lines from *G. australe* in *G. hirsutum*, Sarr et al. (2011) reported a chromosome transmission rate of 100% for Chromosome $10G^a$ to 34% for chromosome $12G^a$. For Chen et al. (2014) the highest incidence for an alien chromosome was 91.32 % for chromosome$10G^a$ and the lowest one was 1.37% for chromosome $5G^a$.

Cytological analysis on chromosomal configuration at meiosis revealed multivalent associations in MAALs PMC. Indeed, the allohexaploid ($2n = 78$, $2(A_hD_hF_1)$) presented tri-, quadri- and pentavalents, indicating that homoeologous recombination should happen between the F and AD chromosomes at the hexaploid and pentaploid stages at a high or low frequency (Koto, 1983).

Phylogenetic analysis suggested that the F genome of *G. longicalyx* is close to the A genome (Cronn et al., 2002). The affinities existing between the F-genome and the D_h subgenome chromosomes were low (21.6 univalents per cell according to Endrizzi et al. (1985). Recombination leading to introgression should have occured with the A subgenome chromosomes.

The amplification rate of *G. hirsutum* microsatellites in *G. longicalyx* (60%) provides evidence of the wide conservation of sequences between the A genome of *G. hirsutum* and the F-genome of *G. longicalyx*. This amplification rate between *G. australe* and *G. hirsutum* was 56% (Sarr et al., 2011) and 66.2% (Chen et al., 2014). The allohexaploid and the MAALs were found to be missing a specific locus of *G. longicalyx* BNL2589 (C11-C21). The elimination of some DNA fragments may have occurred during colchicine diploidization or backcrossing. This phenomenon has been reported previously in cotton (Jiang et al., 2000), wheat (Shaked et al., 2001) and tobacco (Skalicka et al., 2005).

We noticed that BNL4030 and CIR222 mapped in the linkage group C4-C10 were highly transmitted. These findings may indicate that these alleles are transmitted or recombined preferentially in the background of *G. hirsutum*. Chromosome preferential transmission or elimination was observed in various studies on *Gossypium* (Lopez-Lavalle & Brubaker, 2007; Ahoton et al., 2004; Benbouza et al., 2008; Sarr et al., 2012).

Chromosome transmission frequency is known to differ among chromosomes. This variation can be caused by differences in chromosome size or structure or by the presence of genes causing segregation distortion (Diouf et al., 2014).

We noticed that MAALs considered different on the basis of their phenotypes carried the same extra chromosome of *G. longicalyx*. This can be be explained by the simultaneous presence of introgressed fragments of other chromosomes of the wild species in the same G. hirsutum background (Table 5). Phenotypic variation can be explained by several mechanisms such as the lost, mutation or divergence of a gene, chromosomal breakages and rearrangements. Sequence elimination have been reported in wheat and *Tragopogon* allopolyploids (Shaked et al., 2001; Tate et al., 2006), in cotton (Sarr et al., 2012); chromosomal translocations and transposition was observed in *Brassica* allopolyploids (Song et al., 1995); and changes in gene expression

appear to be a major consequence of phenotypic variation in *Arabidopsis* and cotton (Lee et al., 2001; Wang et al., 2004).

We identified four MAALs that will serve to achieve chromosome specific introgression.

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