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Abstract	The efficiency of using monosomic alien addition lines (MAALs) to introgress agronomical traits of interest carried by wild diploid *Gossypium* species into the main cultivated cotton species *G. hirsutum* depends on the opportunities of confronting the alien chromosome and the recipient background genome at each generation and on the occurrence of translocations and homoeologous recombinations. The selfed-progeny of five MAALs of *G. australe* in *G. hirsutum* was screened with SSR markers to determine the transmission frequency of the alien chromosome and monitor its integrity. Three MAALs revealed a transmission frequency significantly lower than the expected ratio and one MAAL presented an exclusive transmission of the additional chromosome. In these four MAAL the alien chromosome was transmitted almost unaltered. With the fifth MAAL the alien chromosome was normally transmitted but was altered in half of the plants containing it. In one MAAL normally carrying brown fiber, the emergence of some plants carrying white and brown fiber revealed the somatic elimination of the additional chromosome. The loss of this chromosome seems to be triggered by its deletion.

Keywords (separated by '-')	Chromosome elimination - Cotton - *Gossypium* - Monosomic alien addition line
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Alien chromosome transmission and somatic elimination in monosomic addition lines of *Gossypium australe* F. Muell in *G. hirsutum* L.

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Keywords Chromosome elimination · Cotton · *Gossypium* · Monosomic alien addition line

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Introduction

Upland cotton (*Gossypium hirsutum* L.), the world's leading natural fiber crop, is one of the five allotetraploïd species ($2n = 4\times = 52$) of a genus which also includes approximately 45 wild diploid species ($2n = 2\times = 26$) (Wendel et al. 1992). The use of monosomic alien addition lines (MAALs) is one of the strategy developed to transfer important agronomic traits carried by these wild diploid species into the main cultivated species (Stewart 1995; Brubaker et al. 1999; Ahoton et al. 2003; Mergeai 2006).

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By limiting the donor genome to a single chromosome, MAALs provide an effective way to identify interesting genes carried by the wild species and to achieve their accurate transfer to produce introgressed plants in a relatively short time (Hau 1981). Comparable to a dissection of a diploid genome in the same genetic background, MAALs constitute as well a very useful genetic material to investigate genome structure and phylogenetic relationships (Brubaker and Brown 2003).

Introgression of desirable genes from the alien chromosome to the cultivated species can be achieved by use of ionizing radiation, tissue culture or chemical mutagens to induce heterogenetic translocations (Jiang et al. 1994; Sears 1993; Lapitan et al. 1984). However, these techniques may also cause great rearrangements and aberrations in the chromosomes of the genetic background resulting in production of genetically non-compensated individuals (Konan et al. 2009). Spontaneous translocations arising from a centromeric or non-centromeric breakage and reunion is theoretically another mechanism allowing the transfer of alien chromosome fragments (Walters 1950). But as translocations induced by irradiation they most likely involve non-homeologous chromosomes and are therefore associated with genetic imbalance due to duplication of the genes brought by the alien segment and deficiencies of the genes lost by the recipient genome. Contrary to spontaneous or induced translocations, homoeologous recombination results in an exchange of a similar-size fragment between the alien chromosome and a homeologous chromosome of the recipient genome and is therefore genetically compensated. In a species like wheat that possesses a meiotic pairing genetic system (Chen et al. 1994; Jiang et al. 1994), chromosomes can be "engineered" to induce homoeologous recombination and minimize the linkage drag. In the genus *Gossypium,* which lacks such a system, the only available solution to induce heterogenetic associations has been to proceed by repeated selfing in order to multiply meiotic events confronting the donor chromosome and the recipient genome (Stewart 1995; Mergeai 2006). In this perspective, identification of the rare recovered recombinants, with a high-throughput procedure is of a major importance. But until recently detection of the recombinants has been based on the time-consuming and painstaking classical cytogenetic techniques combined with analysis of the plant morphology (Rooney et al. 1991). Molecular markers such as SSR, owing to their PCR-based technique, wide distribution in the genome and cost-effectiveness are very useful in overcoming these impediments.

Previous analyses of alien addition stocks issued from the backcrossing to *G. hirsutum* of the [2 (*G. hirsutum* × *G. australe*) × *G. hirsutum*] pentaploid allowed the isolation of 11 MAALs of *G. australe* in *G. hirsutum* out of the possible 13 MAALs (Ahoton et al. 2003; Sarr et al. 2011). The primary objective of this study was to investigate with SSR markers the transmission frequency and integrity of the alien supernumerary chromosome carried by five of the isolated MAALs.

Materials and methods

Plant material

The self-progeny of five MAALs of *G. australe* in *G. hirsutum* was grown in pots in the tropical greenhouses of Gembloux Agro-Bio Tech. They carried the *G. australe* chromosomes homoeologous to the *G. hirsutum* chromosome pairs c2–c14, c3–c17, c6–c25, c10–c20 and c12–c26. (Ahoton et al. 2003; Sarr et al. 2011). The MAALs have been identified from the derived backcross progenies of the allohexaploid between *G. hirsutum* and *G. australe* by cytological (genomic in situ hybridization) and molecular markers (SSR) analyses (Sarr et al. 2011). In this paper these five MAALs will be designated by the combination of the prefix MAAL with the chromosome number of the *G. australe* chromosome (e.g., the MAAL carrying the *G. australe* c10 homoelogous to *G. hirsutum* chromosome pair c10–c20 will be designated MAAL-10) (Sarr et al. 2011). Each of the MAALs was characterized by a more or less marked altered morphology when compared to *G. hirsutum*. MAAL-6 was particularly easily identified: the alien chromosome turned the white-fiber of the *G. hirsutum* cultivar into brown color (Sarr et al. 2011). The self-progeny of each MAAL came from a unique plant. Self-pollination was forced by clipping the flower bud at candle stage.

SSR genotyping

The self-progenies of the five MAALs were investigated with SSR markers specific to the involved

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G. australe chromosome. The number of plants analyzed for each MAAL varied between twenty and ninety-eight. DNA extraction was carried out on sampled leaves of 30-day-old seedlings according to Benbouza et al. (2006a). PCR amplification, gel electrophoresis and silver staining detection were performed according to Benbouza et al. (2006b). The SSR linkage groups characterizing each of the *G. australe* chromosome were established from the progeny analysis of the [2(*G. hirsutum* × *G. australe*) × *G. hirsutum*] pentaploid (Sarr et al. 2011) and are given in Table 1. Sequences of all these markers are available on the website http://www.cottonmarker.org. Out of the ten markers characterizing *G. australe* chromosome c6 only seven have been used in this study to screen the self-progeny of MAAL-6 (Table 1).

Statistical tests

χ^2 Tests were performed to detect bias from the 3:1 expected ratio in the alien chromosome transmission.

Results

Alien chromosome transmission in the five self-pollinated MAALs

Taking into account all plants carrying a supernumerary *G. australe* chromosome, whether altered or not, the alien chromosome transmission frequency of all the MAALs but MAAL-2 ($\chi^2 = 0.6$; $P = 0.438$) were significantly different from the 3:1 expected ratio. But while MAAL-10 showed exclusive transmission of the supernumerary chromosome, MAAL-3, MAAL-6, and MAAL-12 presented an alien chromosome transmission frequency lower than the expected ratio (Table 2). Considering that each SSR linkage group defines a complete *G. australe* chromosome, only seventeen plants of MAAL-2 (38%) and two plants of MAAL-6 (2%) carried altered *G. australe* chromosomes (Table 2).

Somatic elimination of the supernumerary chromosome in MAAL-6

The self-progeny plants of MAAL-6 that did not carry any of the alien chromosome markers produced only bolls with white-fiber. Except for one plant (#13), all the plants carrying the entire markers specific to the alien chromosome produced only bolls with brown fiber. The plant #13 gave brown-fiber bolls *and* white-fiber bolls (Fig. 1). Of the two plants (#37 and #39) carrying altered *G. autrale* chromosomes (plants not carrying all the markers characteristic of the *G. australe* chromosome), one plant (#37) produced only white-fiber bolls while the other (#39) produced white-fiber bolls and brown-fiber bolls.

Table 3 gives the relationships between the fiber color and the number of the *G. australe*-specific loci markers in the 98 self-progeny plants of MAAL-6.

The production of fiber of different colors by plants #39 and #13 was surprising as well as the exclusive production of white-fiber bolls by plant #37. The hypothesis of the elimination of the alien chromosome was tested. The three plants #13, #37

Table 1 SSR linkage groups characterizing the *G. australe* chromosomes (Sarr et al. 2011)

MAAL-2	MAAL-3	MAAL-6	MAAL-10	MAAL-12
CIR202 (195)[a]	MUSS073 (210)	BNL1440 (210)	NAU1182	BNL1679 (137)
BNL3989 (245)	MUCS620 (237)	BNL1061 (155)	CIR187 (290)	BNL3537-2 (170)
JESPR231	CIR180	BNL1047 (155)	CIR171 (218)	BNL1673 (185)
BNL1897 (118)	BNL2443 (124)	BNL3436 (190)	BNL3563 (210)	BNL3599 (175)
CIR228 (192)	BNL3413 (112)	BNL3359 (187)	BNL119	BNL3261(190)
CIR210 (102)		BNL2569 (152)		
CIR292 (202)		BNL3103 (180)		
BNL3259 (198)		BNL3594		
		CIR407 (157)		
		BNL2884 (175)		

[a] Number in parenthesis gives the *G. australe*-specific fragment size in base pairs

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Table 2 Transmission frequency and integrity of the alien chromosome in the five self-pollinated MAALs

	MAAL-2	MAAL-3	MAAL-6	MAAL-10	MAAL-12
Number of plants without the alien chromosome	9 (20)[a]	19 (51)	52 (53)	0 (0)	57 (66)
Number of plants with the complete alien chromosome	19 (42)	18 (49)	45 (45)	20 (100)	30 (34)
Number of plants with an altered alien chromosome[b]	17 (38)	0 (0)	2 (2)	0 (0)	0 (0)
Total number of plants	45	37	98	20	87

[a] Number in parenthesis indicates the percentage

[b] An altered alien chromosome does not carry all the markers characteristic of the *G. australe* chromosome

Fig. 1 Self-progeny plant #13 of MAAL-6 carrying white-fiber and brown-fiber bolls

Table 3 Relationships between fiber color and presence of *G. australe*-specific loci markers in the 98 self-progeny plants of MAAL-6

Plants	Number of markers	Color of the fiber
52 Plants	0 Marker	White
43 Plants	All markers	Brown
Plant #13	All markers	White and brown[a]
Plant #37	1 marker	White
Plant #39	3 markers	White and brown[a]

[a] All the seeds of a boll carry fibers of the same color

and #39 were ratooned to produce new fresh leaves that were sampled on different branches for DNA extraction and SSR analysis. The results of this analysis combined with the fiber color of the bolls are given in Table 4.

All the samples from plants #13 and #37 gave results identical to the initial analysis before ratooning while some branches of plant #39 lost the *G. australe*-specific marker (Fig. 2).

After ratooning the plants #13 and #37 produced only bolls containing white-fiber as the branches of plant #39 that lost all the *G. australe* markers. The two branches of plant #39 that retained the *G. australe* markers carried white-fiber and brown-fiber bolls (Table 4).

The systematic absence of brown-fiber bolls on branches of plant #39 without any marker revealed clearly that the appearance of the white-fiber character was due to the elimination of the alien chromosome. The exclusive production of white-fiber by plants #13 and #37 despite the presence of all the markers is comprehensible if one keeps in mind the fact that, due to the very late chromosome elimination, the genotype of the leaves does not necessarily reflect the genotype of the fibers which are epidermal cells of the seed-coat developing later after leaves have been sampled (see discussion below).

Since the low number of bolls carried by the plants grown in pots (1–3 bolls per branch) (Table 4) did not allow a real understanding of the pattern of the supernumerary chromosome elimination, a total of 104 plants of MAAL-6 self-progeny were grown in the field during the summer in Senegal (West-Africa). Out of the 104 plants, only two revealed mosaicism. With branches much longer and carrying more bolls than branches of the plants grown in pots, we observed that some branches carried white-fiber and brown-fiber bolls while other branches carried white-fiber bolls or brown-fiber bolls only.

Considering two consecutive branches the first-produced could carry white-fiber bolls while the later-produced branches carried brown-fiber bolls. Considering also two successive bolls within a branch, the first-produced could carry white-fiber bolls whereas the second carried brown-fiber bolls. From these observations we could conclude that the chromosome elimination, either inter-branch or

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Table 4 *G. australe*-specific loci markers and fiber color in three self-progeny plants of MAAL-6

	Plant #13[b]	Plant #37[b]	Plant #39[b]	Branches of plant #13[c]						Branches of plant #37[c]			Branches of plant #39[c]					
				1	2	3	4	5	6	1	2	3	1	2	3	4	5	6	7
Markers[a]																			
BNL3436	+			+	+	+	+	+	+										
BNL3359	+		+	+	+	+	+	+	+										
BNL2569	+		+	+	+	+	+	+	+										
BNL3103	+	+	+	+	+	+	+	+	+	+	+	+	+			+			
BNL3594	+	+	+	+	+	+	+	+	+	+	+	+	+			+			
Number of bolls with…																			
White fiber	1	3	3	3	0	2	2	1	0	2	2	0	3	1	0	1	0	0	
Brown fiber	4	0	3	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	

[a] Only half of the 10 markers identifying *G. australe* c6 of MAAL-6 have been used to screen the branches of the plants #13, #37 and #39

[b] Constitution of the plant before ratooning

[c] Constitution of the plant after ratooning

[d] The sign "+" indicates presence of the *G. australe*-specific fragment

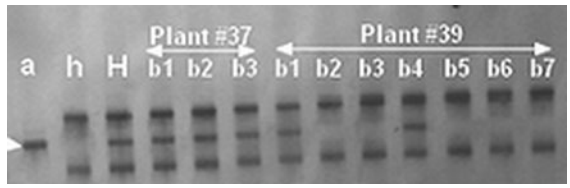

Fig. 2 Loss of the *G. australe*-specific fragment produced by the marker BNL3103 in the self-progeny plant #39 of MAAL-6. *Note*: a = *G. australe*, h = *G. hirsutum*, H = Hexaploid, b = branch. The *arrow* indicates the *G. australe*-specific fragment

intra-branch, occurred completely at random. Moreover, one of the two mosaic plants observed in Senegal produced one boll containing seeds with white-fiber and seeds with brown fiber. However, all the seeds in a loculus carried fiber of the same color (Fig. 3).

Discussion

Alien chromosome transmission in self-pollinated MAALs

Alien chromosome transmission in the self-progeny of MAAL-3, MAAL-6 and MAAL-12 was significantly lower than the expected 3:1 ratio. Distortion in the segregation ratio of MAALs in the genus *Gossypium* is a rather common observation. Rooney and Stelly (1991) found in the self-progeny of 3 MAALs of *G. sturtianum* in *G. hirsutum* an average alien chromosome transmission of 23%. Mergeai (1992) reported transmission frequencies of 52 and 53.8% in two MAALs involving the diploid species *G. areysianum.* It is also well-known that self-pollination in the genus *Gossypium* is likely to overestimate the bias of the alien chromosome transmission because of the differential ability to transmit the alien chromosome between the male and the female gametes on the one hand and because of the strong selection against disomic alien addition lines with the same chromosome in duplicate on the other hand (Hau 1981). Female gametes are better suited for transmission of the alien chromosome than male gametes. Poisson (1970) reported transmission frequencies by the female gamete ranging from 26.2 to 39.2% for six MAALs of G. *anomalum* in

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Fig. 3 Boll with white-fiber and brown-fiber seed-cotton produced by a self-progeny plant of MAAL-6

G. hirsutum and from 0 to 5.89% by the male gamete for the same MAALs. The four MAALs of *G. sturtianum* in *G. hirsutum* studied by Rooney and Stelly (1991) transmitted the alien chromosome exclusively by the female gamete. The two MAALs described by Mergeai (1992) transmitted the alien chromosome through the male gamete at a rate of 2.6 and 6.2% only. The lower transmission of the alien chromosome by the male gamete is usually attributed to a lesser viability and/or competitiveness of male gametophyte with an additional chromosome in the pollen tube growth. On the contrary to the three previous MAALs, MAAL-10 showed an exclusive preferential transmission of the supernumerary chromosome. This preferential transmission was already observed by Rooney and Stelly (1991) in one MAAL of *G. sturtianum* in *G. hirsutum* with a transmission rate through the male gamete of 90%. Becerra Lopez-lavalle and Brubaker (2007) also identified a *G. australe* chromosome designated Aust-M (which could be the same than the one carried by our MAAL-10) transmitted at a rate of 100% by the female gamete. Preferential transmission of a supernumerary chromosome in MAALs was also observed in other species. Cameron and Moav (1957) described a MAAL of *Nicotiana plumbaginifolia* in *N. tabacum* whose alien chromosome was transmitted at 100% by the male gamete; alien chromosomes of *Aegilops sharonensis* and of *A. longissima* in the wheat background were reported to be selectively retained in wheat by both male and female gametes (Maan 1975; Miller et al. 1982). Maguire (1963) reported a MAAL of *Tripsacum* in corn whose alien chromosome was transmitted at 91% by the female gamete.

If a low transmission of the alien chromosome can be explained by prezygotic (meiotic irregularities resulting in the loss of the alien chromosome, unviability and lack of competitiveness of the male gamete) or postzygotic phenomena (less viability of zygotes or plants with an alien chromosome) (Rooney and Stelly 1991), other mechanisms such as apomixis, preferential segregation or gametocidal genes are put forward to explain preferential transmission of the alien chromosome (Rhoades 1942; Maguire 1963; Miller et al. 1982; Gao and Jung 2002). Apomixis has been reported to occasionally occur in hybrids of *Beta vulgaris* and species of the section Corrolineae. It has been assumed to cause the transmission of the alien chromosome in a MAAL of *B. corolliflora* in *B. vulgaris* at a rate of 60% through the female gamete (Gao and Jung 2002). Preferential segregation was described by Rhoades (1942) as the passage of a specific chromosome to a specific pole. He found that the abnormal maize chromosome 10 with an extra piece of chromatin attached to the end of the long arm, observed in some strains grown in the south-western of the USA, was transmitted at more than 70% in the ovules. He explained this distorted ratio by the fact that the abnormal chromosome passed preferentially to the lower pole of the spindle from which the basal megaspore that gives the embryo sac arises while the three other megaspores degenerate. Gametocidal chromosomes carry genes that make non-functional the gametes not carrying them (Cameron and Moav 1957; Jiang et al. 1994; Nasuda et al. 1998). Numerous *Aegilops* species (*A. cylindrica, A. triuncialis, A. sharonensis* etc.) are known to carry these genes. The *G. australe* chromosome designated Aust-M by Becerra Lopez-lavalle and Brubaker (2007) is suspected to carry gametocidal genes as it could also be the case with the alien chromosome carried by MAAL-10. If that turned out to be true, a powerful means will be at the disposal of cotton breeders to harness *G. australe* for the improvement of the main cultivated species. Gametocidal chromosomes from *Aegilops* species for example have been used for mapping as an alternative to radiation (Masoudi-Nedjad et al. 2005) and for introgression

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in wheat (Shi and Endo 1999; Shi and Endo 2000; Masoudi-Nedjad et al. 2002).

Determination of the factors causing the distorted transmission in MAAL-3, MAAL-6, and MAAL-12 will require to test the progeny obtained by back-crossing to *G. hirsutum* as male and female parent and to carry out cytogenetic studies.

Integrity of the alien chromosome in the self-pollinated MAALs

Of the five MAALs, only MAAL-2 and MAAL-6 carried altered chromosomes that could be identified using SSR markers. The fragments carried by MAAL-6 self-progeny seemed not to be recombined (see discussion on the somatic elimination). As for the fragments carried by the MAAL-2 self-progeny, one can suppose that the altered chromosomes observed in 38% of the plants are not recombined since *G. australe* belongs to the tertiary gene-pool of the cultivated species which is characterized by the lowest level of homoeology and pairing affinity with *G. hirsutum* chromosomes (Stewart 1995). Genomic in situ hybridization (GISH) analysis of these plants will be necessary to confirm this hypothesis.

Somatic elimination of the supernumerary chromosome in plant #39 of MAAL-6

Fiber color mosaicism and loss of the *G. australe*-specific loci markers in some leaves in plant #39 of MAAL-6 clearly revealed an elimination of the *G. australe* chromosome. Somatic elimination of the supernumerary chromosome has been already reported by Rooney et al. (1991) in a monosomic addition line of *Gossypium sturtianum* in *G. hirsutum.*

Complete or partial uniparental chromosome elimination following an interspecific hybridization, whether by sexual or somatic fusion hybridization, is a frequently observed phenomenon which has even been used to produce haploid or aneuploid plants (Koba et al. 1991; Riera-Lizarazu et al. 1996; Kynast et al. 2001). But usually it is during the first zygotic divisions of the hybrid embryo development, that the chromosome elimination takes place. For example in wheat and maize hybrids, all maize chromosomes are eliminated during the first three-cell division cycles in the embryo (Laurie and Bennett 1989); in hybrids between wheat and pearl millet, all pearl millet chromosomes are eliminated between 6 and 23 days after pollination (Gernand et al. 2005). When the chromosomes are lost in the early stages of the embryo development, the derived-plant can grow with the same number of chromosomes in all its cells. The very peculiarity of the chromosome elimination reported here is its late occurrence, during the adult plant growth.

After ratooning the fragment carrying the markers BNL2569 and BNL3359 is lost in all analyzed branches while the fragment with the markers BNL3103, BNL3594, CIR407, and BNL2884 was still retained in some branches. This observation seems to reveal that the first fragment was less stable than the second.

Production of some brown-fiber bolls on these branches indicates that the gene responsible of the brown color of the fiber is located on the retained fragment carrying the markers BNL3103, BNL3594, CIR407, and BNL2884.

The apparent inconsistency observed between the results obtained with the SSR markers and the color of the fiber in the plants #13 and #37 of MAAL-6 has to be interpreted considering the results obtained from plant #39. The important conclusion that can be drawn from these results is that the chromosome elimination can occur at any moment in the growing plant.

Because the chromosome elimination can occur at any moment a correlation may not exist between the genotype of the leaves that gave the DNA analyzed with the SSR markers and the other parts of the plant, notably the fiber constituted by epidermal cells of the seed-coat. This discrepancy is all the more likely since fibers are among the latest produced cells.

The plant #37 carried only white-fiber bolls, despite the fact that it carried the two same *G. australe*-specific SSR loci as the branches of the plant #39 that gave some brown-fiber bolls. The number of four SSRs being too low to characterize satisfactorily a chromosome fragment it is likely that the fragments present in the two plants carry the same two markers but are not identical. Two hypotheses could be put forward : (i) the chromosome elimination in plant #37 is more severe in the fibers than in the leaves or (ii) the chromosome elimination severity is the same whatever the type of cell, but the fragment carried by the plant #37 has in fact lost the

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region carrying the gene. Considering the pattern of the chromosome elimination in the plant #39, instability in leaves being accompanied by instability in the fibers, there is no reason to think that the chromosome elimination varies according to the type of cell. So the persistence of the four markers BNL3103, BNL3594, CIR407, and BNL2884 in the leaves of plant #37 combined with the regular production of white-fiber favored the second hypothesis: although plant #37 and the two branches of plant #39 carry the four same *G. australe*-specific loci, they carry in fact two different fragments. The fragment carried by plant #37 likely lost the gene responsible of the fiber brown color and did not undergo somatic elimination. As for the fragment carried by plant #39 it carried the gene responsible of the fiber brown color and did undergo somatic elimination. Thus, there seems to be a variation in the level of stability according to the nature of the *G. australe* fragment.

Timing, origin and mechanisms of the chromosome elimination

The configuration of a mosaic formation due to chromosome elimination allows the determination of the timing of the chromosome loss. Since a sector is constituted by a cell lineage originating from the same cell, the size of a sector depends on the moment when the chromosome is eliminated. The largest a sector is, the earliest the chromosome has been eliminated. Based on this principle, Rooney and Stelly (1991), observing no intra-branch mosaicism, concluded that somatic elimination of the supernumerary chromosome happened at a precocious stage, during the formation of the branches developed from a single adventitious bud. In the case studied here, the existence of inter-branch, intra-branch and even an intra-boll mosaicism revealed clearly that the chromosome can be lost at every moment, even until flowering, in the growing plant.

It is most likely that the *G. australe* chromosome elimination has been triggered by its previous breakage. Correlation between chromosome breakage and instability is a well-known fact. McClintock (1941) described how breakage of a single chromatid results, after division, in the fusion of the two sister halves of the broken chromosome creating a dicentric bridge that will break during poleward migration in the following mitotic anaphase. This breakage-fusion-bridge (BFB) sequence will continue in each successive nuclear division until the broken chromosome end is healed. This BFB cycle can occur at meiosis as well as in mitosis of somatic cells. An important question to be answered in the present study, because it would help understanding the mechanisms of the somatic elimination, is to determine whether the breakage of the chromosome undergoing elimination occurred after fertilization in the sporophytic tissues (maybe even beginning in the embryo) or before (during gametogenesis). In the first case, the chromosome is not expected to be healed. Though healing of broken chromosome ends in the sporophytic tissues is not impossible, it is very rare and is thought to occur only in certain physiological conditions (McClintock 1941). It is also known that breaks induced by mechanical rupture, as it is the case here, and by X-ray, contrary to those caused by ultraviolet radiation, are followed by fusions of broken ends of the chromosomes (McClintock 1941). So in the present case the mosaicism could be explained by instability due to the BFB cycle. But this explanation should result in a high-intensity of variegation (Moav 1961) and for example more intra-boll and even intra-loculus variegation should be observed. In the second case the centric fragment of the broken chromosome is not expected to undergo a BFB cycle but should be transmitted normally like a complete chromosome because the broken end of a chromosome is known to be permanently healed after its passage in the embryo. So if the broken chromosome is unstable in the sporophytic tissues causing mosaicism, it is more likely because the chromosome fragment undergo rare aberrant mitotic anaphase (non-disjunction of sister chromatids and lagging or migration of both chromatids to one daughter cell) resulting in the deficiency of the fragment in some cell lineages expressed by the mosaicism. Gupta (1968) studying different plants with a chromosome fragment of *Nicotiana plumbaginifolia* in *N. tabacum* found out a frequency of mitotic anaphase bridges formed by the *N. plumbaginifolia* chromosome ranging from 2.6 to 20.9% positively correlated with the intensity of the variegation.

Conclusion

Presence of a dominant marker gene in the *G. australe* chromosome c6 provided an opportunity

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to detect the somatic elimination of the alien chromosome. The frequencies obtained in the greenhouse and in the field are consistent and are around 2% of a self-progeny. Because it reduces the meiotic events confronting the donor chromosome and the recipient genome, the chromosome elimination is an obstacle for the achievement of homoeologous recombination and above all can result in dramatic consequences in terms of waste of time and resources if it goes unnoticed.

So in the breeding programs involving MAALs lacking a qualitative morphological marker much attention will have to be paid to the possible elimination of the alien chromosome.

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