

Segregation Distortions in an Interspecific Cotton Population issued from the [(*Gossypium hirsutum* x *G. raimondii*)² x *G. sturtianum*] Hybrid

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Summary

The segregation ratio of 10 *Gossypium sturtianum* specific SSR markers belonging to linkage groups c2-c14, c3-c17, and c6-c25 was analysed in the BC2S6 progeny of the [*G. hirsutum* x *G. raimondii*]² x *G. sturtianum*] (HRS) hybrid; based on chi-square test. All the marker loci tested were associated with skewed allele frequencies ($P < 0.001$) showing a strong SD with a zygotic selection. The possible causes and consequences of this massive segregation distortion are discussed.

Résumé

Distorsion de ségrégation dans une population interspécifique de cotonnier issue de l'hybride [(*Gossypium hirsutum* x *G. raimondii*)² x *G. sturtianum*]

Le rapport de ségrégation de 10 marqueurs SSR spécifiques de *G. sturtianum* situés sur les groupes de liaison c2-c14, c3-c17, et c6-c25 a été analysé dans la descendance BC2S6 de l'hybride *G. hirsutum* x *G. raimondii*)² x *G. sturtianum*] (HRS) en utilisant le test chi carré. Tous les marqueurs testés ont montré de manière très significative ($P < 0,001$) une distorsion de ségrégation influencée par une sélection zygotique. Les causes et conséquences de cette importante distorsion de ségrégation sont discutées.

Introduction

The cotton genus, *Gossypium* L. (Malvaceae) contains 49 diploid and tetraploid species distributed worldwide in both tropical and subtropical areas. Cotton is the world's leading natural fiber crop. Furthermore, its seed can be used in food and feed. Cotton is the fifth best oil-producing plant in the world and the second best potential source of plant proteins. The ability to use this nutrient-rich source for food is mortgaged by the presence of pigment glands throughout the plant and seeds; which contain toxic terpenoid aldehydes.

Few Australian cotton diploid species produce gossypol free seeds while maintaining a normal density of pigment glands in their areal parts. One of them named *Gossypium sturtianum* Willis (2C1)

was used to introgress the low-gossypol seed and high-gossypol plant trait into *G. hirsutum* tetraploid, 2(AD)h, through the development of the [(*G. hirsutum* x *G. raimondii*)² x *G. sturtianum*] (HRS) trispecific hybrid according to the pseudophyletic introgression method (15, 18).

The transfer of desired genes or gene clusters from alien species to superior cultivars is often accompanied by unacceptable wild traits present in the transferred chromosomal segment. Generally, procedures for chromosome pairing manipulation in polyploid crop plants referred to as 'chromosome engineering', leads to fruitful recombination of entire genomes, genome parts or chromosomal segments. The results of such manipulations are genomic reconstructions leading to a reduction of the size of the alien chromosomal segment transferred to the genome of the recipient species.

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However, in the seeds produced by the selected HRS derivatives, a high segregation of the gossypol content was still observed after more than six generations of backcross and selfing (1). Furthermore, high rates of mortality, empty and malformed seeds were observed (1, 3). These observations are probably due to the maintenance in the heterozygous state of large parts of the HRS hybrid chromosomes; which are often results of Segregation distortion (SD) (11, 17). During interspecific crosses, genes, useful or not, employ various strategies to stay in host organisms (14). SD is one of the main factors that limit the introgression of valuable agronomic traits in a crop from related alien species.

Segregation distortion can be defined as a deviation from the expected Mendelian proportion of individuals in a given genotypic class within a segregating population. Frequently found during the construction of genetic linkage maps, SD has been reported in both animals and plants (17). It was firstly reported in maize and subsequently genomic regions harboring markers with SD have been notified in many crop species including rice, barley, sorghum, and tomato (17). In cotton, segregation distortion has been noticed in intraspecific (10) and in interspecific crosses (17, 20). SD can be detected for almost any kind of markers (19) and seems to be particularly common in wide crosses (17, 19). A variety of physiological and genetic factors can be involved in SD. Xu *et al.* (19) reported several SD mechanisms related to preferential fertilization due to gametic selection (pollen tube competition, pollen lethality), and selective elimination of zygotes. Furthermore, Song *et al.* (17) added that clusters of segregation distortion markers usually result from the selective effect of segregation distortion *loci* (SDL). The following factors can also cause SD: environmental effects (19), a mutation within the binding site for DNA marker, complementary genes, irregular meiosis, heterogeneous recombination, chromosome rearrangement, chromosomal abnormalities (17), and more rarely errors in marker genotyping and statistical analysis.

The aim of the present article is to report the segregation distortion phenomenon in HRS hybrid BC2S6 derivatives, to understand its causes, and to evaluate its consequences for the use of HRS stocks in breeding programs.

Materials and methods

Plant materials

The investigations were carried out on the BC2S6 progeny of the [(*G. hirsutum* x *G. raimondii*)² x *G. sturtianum*] (HRS) trispecific hybrid developed in Gembloux (15). In total, 78 plants obtained by auto-pollinating a HRS BC2S5 individual were screened using ten conserved and specific *G. sturtianum* SSRs markers mapped on c2-c14, c3-c17, and c6-c25 linkage groups of *G. hirsutum* (1).

SSR analysis

Total DNA extraction was performed from young leaves of each plant using the method of Benbouza *et al.* (1). SSR analyses were carried out on the extracted DNA as described by Benbouza *et al.* (1) using a silver staining revelation technique. Mapdisto and Mapchart softwares were used for linkage analysis and map construction. The linkage groups (LGs) were determined using a LOD score threshold of 3.0. The Kosambi mapping function was used for calculation of the pairwise distances (6).

Analysis of segregation distortion of markers

A chi square (χ^2) test was used to compute segregation distortion on the expected Mendelian ratio which is 1:2:1 for co-dominant markers. The markers showing non-Mendelian segregation ($P < 0.05$) were considered to show segregation distortion. Allele frequency 1:1 ($p = q$) and the distribution of different genotype frequencies in this population ($p^2:2pq:q^2$) were analyzed to characterize factors resulting in distorted segregation (12).

When significant deviation from these ratio was observed for a locus, the distortion coefficient, d , was calculated as $d = 1/2 - f(S)$, with $f(S)$ being the frequency of the *G. sturtianum* wild allele, to determine the skewed direction (16).

Results

Segregation distortion in HRS

Genotype segregation for co-dominant markers was analysed against the expected Mendelian ratio (1:2:1) using chi square test. Results showed significant ($P < 0.001$) segregation distortion for the ten tested markers. All of them are skewed towards the heterozygote genotype at 100% except for the SSR BNL 226 which is heterozygote at 94% (Table 1).

Table 1
Chi-square test for segregation distortion of the transferred SSR markers on HRS BC2S6.

Chromosome	Conserved SSR markers	Homozygote <i>G. hirsutum</i> (%)	Heterozygote (%)	Homozygote <i>G. sturtianum</i> (%)	Khi2	Favored genotype
C2	BNL3590	0	76(100)	0	76***	HS
	BNL3971	0	78(100)	0	78***	HS
C3	BNL2443b	0	74(100)	0	74***	HS
	BNL226b	5(6)	72(94)	0	58,95***	HS
	BNL3989	0	76(100)	0	76***	HS
	CIR058	0	70(100)	0	70***	HS
	CIR228a	0	72(100)	0	72***	HS
C6	BNL3359b	0	77(100)	0	77***	HS
C25	BNL3436	0	78(100)	0	78***	HS
	BNL1153	0	78(100)	0	78***	HS

Table 2
Analyse of allele frequency of distorted markers on HRS BC2S6.

Chromosome	Conserved SSR markers	Gene frequency		Khi2		d=1/2-f(B)	Favored allele
		p	q	p2:2pq:q2	p=q		
c2	BNL3590	0.5	0.5	76***	0	0	-
	BNL3971	0.5	0.5	78***	0	0	-
c3	BNL2443b	0.5	0.5	74***	0	0	-
	BNL226b	0.53	0.47	59.36***	0.65	0.03	H
	BNL3989	0.5	0.5	76***	0	0	-
	CIR058	0.5	0.5	70***	0	0	-
	CIR228a	0.5	0.5	72***	0	0	-
c6	BNL3359b	0.5	0.5	77***	0	0	-
c25	BNL3436	0.5	0.5	78***	0	0	-
	BNL1153	0.5	0.5	78***	0	0	-

Analysis of the genetic distortion factors

Allele frequency ($p=q$) and the distribution of different genotype frequencies ($p^2:2pq:q^2$) in the HRS inbreed population were analyzed to find out factors attributed to distorted segregation. All the markers in HRS BC2S6 plants showed distorted genotype frequency, and normal allele frequency (Table 2). These results indicate that the SD occurred mainly at the zygotic level. However, none of the parental alleles are favoured except for BNL 226b which is skewed to the cultivated parent allele H.

Mapping and segregation distortion analysis

The first linkage analysis tests revealed that the ten SSR markers studied in HRS BC2S6 plants were distributed in one linkage group. This result is probably due to the strong distorted segregation, exhibited by all markers and is different from those obtained in previous cotton mapping work. To understand this paradoxical observation it is important to have in mind that the mapping software try to find the best order in long sequences as it is very time and memory consuming to investigate all possible orders with large number of loci. This means that it does not provide the best order (according to the chosen criteria) with certainty. However, it should always give an order

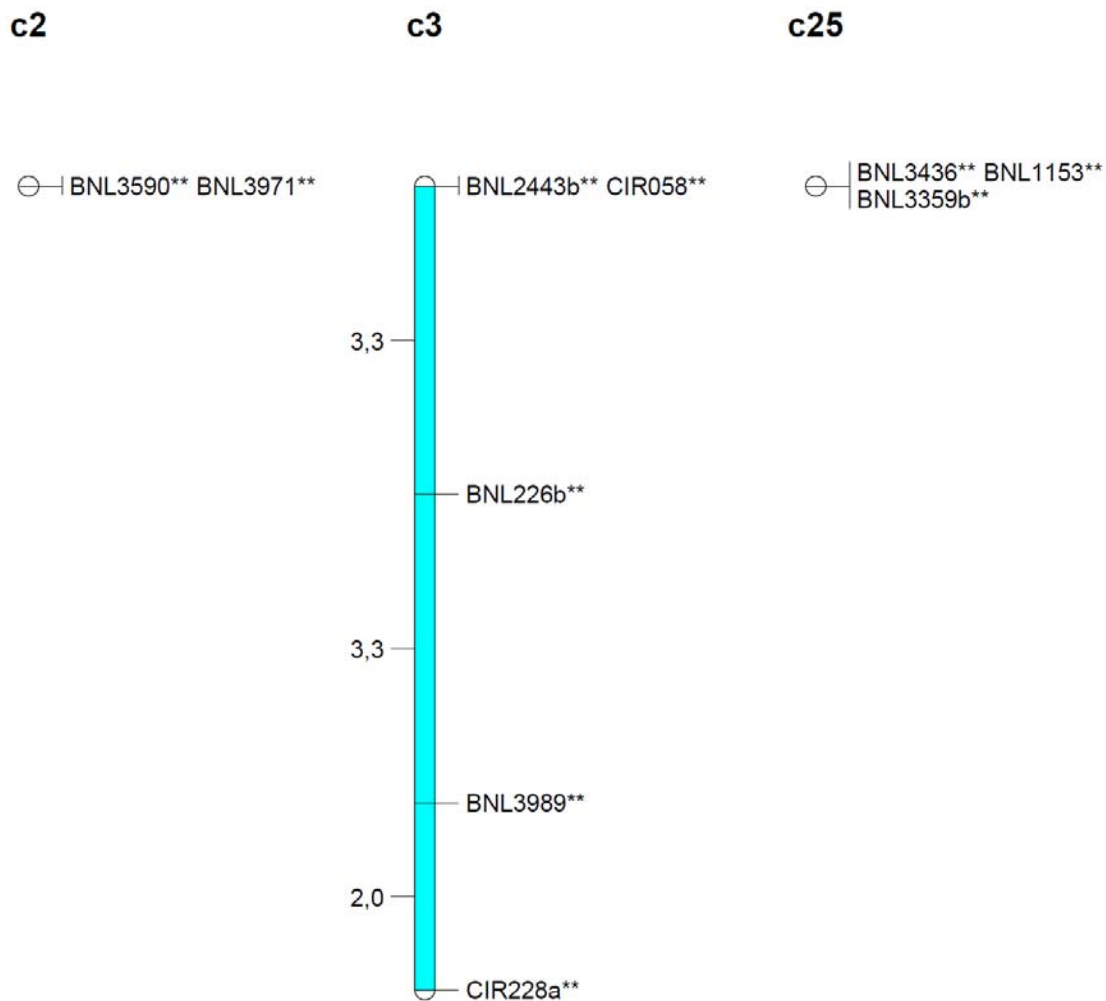


Figure 1: Distribution of molecular markers on target linkage group on HRS BC2S6 (78 individuals). Markers showing segregation distortions are indicated with stars. Numbers in the left side represent linkage distance in centimorgan (cM).

that is close to the best one. All the tested markers were employed in previous studies for mapping populations in cotton and were known to be located on linkage group c2-c14 for BNL3590 and BNL3971, on linkage group c3-c17 for BNL2443b BNL226b BNL3989, CIR058 CIR228a, and linkage group c6-c25 for BNL3359b, BNL3436 and BNL1153 (7, 20). Considering the probable conservation of the precise order of genes on a chromosome transferred from a common ancestor (sharing synteny) and the available comparative molecular marker maps developed in cotton, we imposed the order of markers to follow and to estimate the distance covered by each set of SSR markers corresponding to the three identified linkage groups. The results showed that for the HRS BC2S6 population, the distance between two

successive SSR markers located on chr 2 and chr 6 is nil (total distance = 0 cM), and it is very short on chr3 (between 2 cM to 3,3 cM), with a total length of 8,6 cM (Figure 1). These results supposed that the SSRs BNL3359b, BNL3436 and BNL1153 on linkage group c6-c25 should be tightly linked, as it must also be the case for BNL3590 and BNL3971 on linkage group c2-c14. These results are not coherent with the data available from the existing cotton genetic maps (7, 20). They presumed the existence of entire chromosome (or long chromosome fragments) from each of the two parents between which recombination has not occurred during eight generations of backcross and selfing. This observation is in agreement with a preferential transmission of very long chromosome fragments of *G. sturtianum* in the HRS hybrid.

Discussion

In this cotton breeding program, all the studied population showed strong SD with a zygotic selection due to genetic factors specific to HRS hybrid.

The most reported genetic cause of distorted segregation in crops concern gametophytic factors acting on male, female or both germ lines (13, 19). Generally, mechanisms inducing gametic selection only operate in pollen. However, zygotic selection exists and does not exclude gametic selection, as observed in coffee (9) and in a population of *G. hirsutum* x *G. barbadense* interspecific cross in cotton (17). Both gametic and zygotic selections give rise to most of the distorted segregation in plants (14). They may be controlled by a Segregation Distortion Locus (SDL) with selective effect, depending on the time of their action, before and after fertilization, respectively (17). If they are expressed before fertilization, SDLs can change only the genotypic ratio of zygotes indirectly, by altering the gamete ratio. If SDLs are expressed after fertilization, they affect the genotypic ratio of zygotes directly, as found in this study. Also SDLs can affect chronically segregation in some chromosomal regions. Indeed, there are regions where many distorted loci are distributed in clusters and mostly skewed in the same direction. They constitute hot Segregation Distortion Regions (SDR) (19). Previous studies (11, 13, 17) showed that SDR is usually related to segregation distortion loci (SDL). In cotton, SDR were already found on our targeted chromosomes; c2, c3, c6, c25 (4, 5, 17, 20). All the tested markers showing aberrant segregation in HRS hybrid were thus located in putative SDRs.

Previous research works indicated that SD can be related to the genetic background of both parents was prone to happen in interspecific population (10). It is known that the transfer of desired genes or gene clusters from alien species to superior cultivars is often accompanied by unacceptable wild traits due to “bad” genes also present on the transferred chromosome segment. Genetic incompatibilities, competition among gametes and lethal genes could give rise to abortion of the gametes or zygotes (14, 19). Due to this fact, it is difficult to obtain stable progeny lines or the transferred character can be lost during backcross generations in some populations derived from interspecific crosses.

The Australian species *G. sturtianum* (genome C) belongs to tertiary gene pool of *G. hirsutum* and is the most difficult diploid genome used for gene introgression into domesticated cotton (18). The results we obtained are in conformity with those of Benbouza *et al.* (1), and Vroh Bi *et al.* (18) who carried out molecular analysis on the early backcross and selfing generations of HRS. They revealed that the introgressed progenies were instable and heterozygous for numerous parental alleles.

The major problem in the context of the introgression of desirable traits of wild species to a cultivated species is often the effect of a locus subject to selection by large foreign chromosomal fragments, even to one or more whole chromosomes. This favours a structural heterozygosity which can influence the high rate of heterozygous genome in interspecific hybridization. Lyttle (14) reports that in many plant and animal species, structural heterozygosity can favor specialized parasitic chromosomes. The possible presence of whole or large segments of *G. sturtianum* chromosome in tested HRS progeny, which play a role of “ultraselfish” elements as describe by Lyttle (14) might explain the very high level of zygotic selection observed in HRS progeny.

This situation has important implications on the development of linkage maps. According to Cloutier *et al.* (2), residual heterozygosity in parental lines has yielded subsequent difficulties in linkage determination. SD is one of the factors influencing the precision of distance between markers and order of markers on linkage groups (11, 12, 17). If SD is caused by a SDL, all markers in the vicinity will be affected (11). It also influences QTL analysis which could be negligible or imprecise at best, according to that the recombination frequencies are inferred incorrectly (11, 17). In fact, data from diploid alfalfa (*Medicago sativa*) showed that SD which extremely favours heterozygous individuals, could artificially link genetic region that turned out to be unlinked (17). Beside this, experimental technique for residual heterozygosity in parental lines has resulted in complicated allelic assignment and has yielded subsequent difficulties in linkage determination (2, 17). Lambrides *et al.* (8) concluded that the test for color in mungbean populations was controlled by a single locus and that the two-gene model suggested by the classical analysis was erroneous due to the SD effects.

Because of the importance of the SD factors existing in the HRS hybrid (100 % of the tested markers are skewed), the mapping results obtained

in the HRS BC2S6 population must be very different from the reality. Considering the shared synteny observed between *G. hirsutum* chromosomes and their homeologs in other diploid species (7, 20), the distance between the SSR loci tested cannot be nil in c2-c14, and c6-c25 linkage groups. These results emphasize the difficulty to break the unfavorable linkages that seem to exist between the genes controlling the “glandless-seed and glanded-plant” trait and those involved in SD.

Conclusion

The results obtained validate the consistent presence of distorted markers on the three tested linkage groups in the HRS BC2S6 progeny. The SD appears to be mostly caused by zygotic selection. This selection can be due to the existence of lethal alleles leading to mortality and/or malformation of HRS seeds and plants when they are present in a homozygous state. The presence of unaltered large chromosome and/or large chromosome fragments of *G. sturtianum* causing structural heterozygosity may also favour SD.

We can thus conclude that the genetic background of HRS hybrid is very propitious to the emergence of segregation distortion. In this context, SD of particular loci or chromosomes could cause serious problems in introgression breeding if they are

closely linked to agronomically important genes, as it seems to be the case for the “glanded-plant and glandless seed” trait. The success of these breeding programs is highly dependent on an understanding of these distorting phenomena and on the ability to control them. Hence, further studies should be carried out to understand the genetic factors causing SD in HRS. Genomic *in situ* Hybridization (GISH) should permit to put in evidence the presence of whole chromosome(s) or chromosomal fragments of *G. sturtianum* that may explain the absence of recombination between alien and *G. hirsutum* SSR alleles in the investigated linkage groups.

It is also important to note that zygotic selection does not exclude gametic selection. This means that the existence of gametophytic factors controlled by SDLs with selective effects on the genetic nature of the gametes may be possible. The development of backcross populations from HRS derivatives using *G. hirsutum* as male and female parent should bring more information on this aspect.

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