

Crossing and Setting of Hybrid Seeds in the Creation of Chromosome-Substituted Cotton Lines *g. Hirsutum* L.

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Abstract. A comparative study of the indicators of crossability and setting of hybrid and backcross seeds F1, F1BC1, F1BC2, obtained from crossings of monosomic and monotelodisomic lines of cotton *G.hirsutum* L. with the donor line Pima 3-79 of the species *G.barbadense* L. and aneuploid backcross hybrids, was found to be a linear decrease of these indicators in some hybrids, as well as their increase, as well as the alternation of indicators of crossability and setting in different hybrid generations. The study of chromosome conjugation in hybrid monosomic F1 obtained from crossing monosomic lines with the donor line Pima 3-79 of the species *G. barbadense* L., as well as backcross monosomic F1BC1, F1BC2 with the replacement of specific chromosomes of the cotton genome, revealed normal chromosome conjugation with the formation of 25 bivalents and one univalent of different size in all studied PMCs in most hybrids. Key words: crossability, hybrid seed setting, monosomic lines, backcrossing, chromosome conjugation, cotton, *G.hirsutum* L.

1 Introduction

Obtaining forms with substituted chromosomes (CS - chromosome substitution) in various plants allows for directed introgression into the genome of the *G. hirsutum* L. species of specific chromosomes or arms of individual chromosomes, which are a valuable source of new gene alleles. Previously, such lines were created in a number of crops, which made it possible to improve some agronomic traits [1-9].

For a number of years, research has been carried out in cotton in the USA on obtaining chromosome-substituted lines with the participation of three tetraploid species (*G. barbadense*, *G.tomentosum*, *G.mustelinum*), and with the participation of *G. barbadense*, 20 lines with substitutions of individual chromosomes have already been obtained [10].

The resulting lines made it possible to find out that the replacement of certain chromosomes of the cotton species *G. hirsutum* L. with chromosomes of the species *G. barbadense* L. (CS-B02, CS-B04, CS-B16, CS-B17, CS-B22Lo, CS-B22sh, CS-B25) affect fiber elongation, fiber yield, fiber strength, micronaire, etc. compared to parental lines TM-1 and Pima 3-79 [11]. Since the creation of these lines was not accompanied by

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molecular genetics analyzes, it turned out that some lines (**CS-B05sh**, **CS-B06**, **CS-B07**, **CS-B15sh**) are characterized by the absence of molecular genetics confirmation [6]. In addition, one of the DNA pools, which was previously considered to be derived from a plant hemizygous for **Chr-17** of the species *G. barbadense*, turned out to be hemizygous for **Chr-11** [12], which requires the creation of new CS-lines according to these chromosomes. Therefore, obtaining new aneuploid cotton lines and creating new chromosome-substituted lines on their basis is relevant and necessary.

The use of various types of irradiation made it possible to create a large collection of cotton plants with various aberrations of individual chromosomes at the National University of Uzbekistan [13]. The use of translocation tester lines with numbered chromosomes made it possible to identify shortages of five nonhomologous chromosomes (**2, 4, 6, 7, 12**) and individual chromosome arms (**6** and **11**) of the A_t subgenome, as well as shortages of four chromosomes of the D_t subgenome (**17, 18, 21, 22**) among monosomic lines. The identification of other lines of our collection with shortages of individual chromosomes continues. We use the identified monosomic lines as a tool for foreign introgression into the genome of the species *G. hirsutum* L. of specific chromosomes or chromosome segments from the species *G. barbadense* L. Such studies required the application of the principles of molecular deletion analysis using chromosome-specific microsatellite markers (SSR), previously assigned to specific cotton chromosomes, when the absence of amplification products of all SSR loci localized on a certain chromosome of the *G. hirsutum* species and the presence of markers of the homeologous chromosome of the *G. barbadense* species indicated the replacement of the corresponding chromosome of the first species. The development and application by us of a new scheme for creating chromosomes of substituted cotton lines using both cytogenetic and molecular markers [4] allowed us to start creating new cotton lines with foreign replacement of specific chromosomes. The purpose of this article is a comparative analysis of the crossability and setting of F_1 hybrid seeds and F_1BC_1 and F_1BC_2 backcross seeds obtained from crossings of identified monosomic lines (with a lack of chromosome **2, 4, 6, 7, 12, 17, 18, 21, 22**) and monotelodisome lines (with a lack of the arm of chromosomes **6** and **11**) of the Cytogenetic collection of the *G. hirsutum* L. species with the Pima 3-79 line of the *G. barbadense* L. species and with interspecific aneuploid hybrids, according to the corresponding chromosomes.

2 Materials and methods

In the process of research, a comparative analysis of the crossability and setting of hybrid seeds obtained from crossings of the identified monosomic lines of the NUUz cytogenetic collection of the species *G. hirsutum* L. with the donor line Pima 3-79 of the species *G. barbadense* L., as well as the corresponding F_1 and F_1BC_1 hybrids, was carried out.

3 Results and discussion

An analysis of the crossability of 31 monosomic lines of the NUUz Cytogenetic Collection of the species *G. hirsutum* L. with the Pima 3-79 line of the species *G. barbadense* L. revealed significant differences between the lines. Thus, four monosomic lines with a lack of chromosome **2** (Mo11, Mo16, Mo19, Mo93) were characterized by a decrease in crossability (from 33,333 to 66,67%).

The crossability of 16 monosomic lines with a lack of chromosome **4** with the Pima 3-79 line differed significantly (from 16,66 to 83,33%), where the lowest crossability (16,66%) was noted in the Mo72xPima 3-79 combination, while in another combination

(Mo59xPima 3-79) is the highest (83,33%). The study of the crossability of monosomic lines with a lack of chromosome **6** also revealed differences (from 26,32 to 60,00%). The monosomic line Mo27 with a lack of chromosome **7** was also characterized by a decrease in crossability up to 50.00%. The two newly identified monosomic lines (Mo94 and Mo56) lacking chromosomes **12** and **17** were characterized by a decrease in crossability (up to 33.33 and 40.00%, respectively), while the lowest crossability (16,66, 16,67 and 14,29%, respectively) was observed when crossing three other monosomic lines (Mo48, Mo42 and Mo17, respectively) with shortages of chromosomes **18**, **21** and **22**.

The crossability of two monotelodisomic lines (Telo12 and Telo21) with lack of chromosome arms **6** and **11** obtained from crossings with the donor line Pima 3-79 also differed significantly (50,00 and 100%, respectively).

The results of the study of the crossability of F₁BC₁ hybrid plants obtained from backcrossing of 26 identified monosomic lines of the cotton species *G. hirsutum* L. (with shortages of chromosomes **2**, **4**, **6**, **7**, **12**, **17**, **18**, **21** and **22**) and two monotelodisome lines (telo 6 and telo 11) with the original F₁ hybrids (MoxPima 3-79) revealed a general decrease in crossability involving 19 monosomic lines (from 7,14 to 64,29%) and two monotelodisome lines (from 33,00 to 80,00%), except for five variants involving monosomic lines Mo75, Mo27, Mo56, Mo42 and Mo17 with the replacement of chromosomes **4**, **7**, **17**, **21** and **22**, respectively, in which an increase in crossability was observed in comparison with the original hybrids (F₁(MoxPima 3-79)).

Study of the crossability of F₁BC₂ hybrid plants obtained from backcrossing of 13 identified monosomic lines of the cotton species *G. hirsutum* L. (with shortages of chromosomes **2**, **4**, **6**, **7**, **12**, **18**, and **22**) and one monotelodisomic line (telo 21) with aneuploid hybrids F₁BC₁, found that the crossability varied significantly (from 7,14% to 100%), and in eight variants of crossings (with lines Mo58, Mo59, Mo75, Mo34, Mo92, Mo27, Mo94 and Mo48) an increase in crossability was observed in F₁BC₂, then as in five variants (with lines Mo16, Mo38, Mo60, Mo17, telo21) - a decrease in crossability.

Thus, over the course of three hybrid generations, four studied variants (with the participation of lines Mo16, Mo38, Mo60 and telo21) experienced a linear decrease in crossability, while two monosomic lines (Mo75 and Mo27) with a lack of chromosomes **4** and **7**, respectively, were characterized by an increase in this indicator. The remaining seven monosomic lines (Mo58, Mo59, Mo34, Mo92, Mo94, Mo48 and Mo17) were distinguished by alternating crossability between F₁, F₁BC₁ and F₁BC₂ hybrids. In general, significant differences in crossability in the studied hybrid variants F₁, F₁BC₁ and F₁BC₂ were due to both the interspecific characteristics of the crossed species and the specificity of the lack of individual chromosomes in the tested monosomic cotton lines, as well as the difficulties of combining the flowering period during backcrossing in monosomic and monotelodisome lines cotton, on the one hand, and aneuploid hybrids of different generations, on the other, since lines with shortages of individual chromosomes or their arms were characterized by reduced flowering rates.

The study of the set of F₀ hybrid seeds obtained from crossings of four monosomic lines with a lack of chromosome **2** (Mo11, Mo16, Mo19 and Mo93) revealed a decrease in seed set (from 35,41±3,31 to 57,69±9,69%), in compared with control (73.83±4.25).

The set of F₀ hybrid seeds obtained from crosses of 16 monosomic lines with a lack of chromosome **4** showed a decrease in a larger range (from 26,03±5,14 to 61,76±8,33%). Thus, the lowest setting (26,03±5,14%) was found in one crossing variant (Mo70xPima3-79), while the highest setting of hybrid seeds (61,76±8,33%) was observed in another crossing variant (Mo73xPima 3-79).

The study of the set of hybrid F₀ seeds obtained from crossings of four monosomic lines with a lack of chromosome **6** (Mo34, Mo67, Mo92 and Mo95) revealed a stronger

decrease in seed set (from $18,64 \pm 5,07$ to $33,33 \pm 4,67\%$), in comparison with the control ($73,83 \pm 4,25\%$).

The monosomic line Mo27 with a lack of chromosome 7 was also characterized by a decrease in the set of F_0 hybrid seeds (up to $39,53 \pm 7,46\%$), while the monosomic line Mo48 with a lack of chromosome 18 was distinguished by a high seed set ($82,61 \pm 7,90\%$). Hybrids with four newly identified monosomic lines (Mo17, Mo42, Mo56 and Mo94) when crossed with the Pima 3-79 line also showed reduced hybrid seed set, because when crossing the monosomic line Mo94, which lacks chromosome 12, and the monosomic line Mo42, which lacks chromosome 21 showed a smaller decrease (to $57,58 \pm 8,60$ and $61,76 \pm 8,33\%$, respectively). The hybrid combination (Mo56xPima 3-79) with the participation of the monosomic line Mo56, which had a lack of chromosome 17, was distinguished by a stronger decrease in the set of hybrid seeds (up to $41,17 \pm 4,22\%$), while the hybrid Mo17xPima 3-79, with the participation of the monosomic line Mo17 with a lack of chromosome 22 was distinguished by the strongest decrease in the set of hybrid seeds (up to $29,41 \pm 7,81\%$), compared with all the studied variants with the participation of new monosomic cotton lines.

The study of the setting of F_0 hybrid seeds obtained from crossings of two monotelodisomic lines (Telo12 and Telo21) with lack of chromosome arms 6 and 11, obtained from crossings with the Pima 3-79 line, revealed significant differences between the lines ($69,05 \pm 7,13$ and $16,00 \pm 4,23\%$, respectively).

The set of F_1BC_1 hybrid seeds was also characterized by a decrease in 17 crossing variants compared to the original F_1 hybrids (MoxPima 3-79 and TeloxPima 3-79), with the exception of seven variants involving the lines Mo11, Mo16, Mo19, Mo31, Mo67, Mo95 and Mo27, in which there was an increase in the set of F_1BC_1 hybrid seeds compared to F_1 hybrids. Unfortunately, in the monosomic line Mo13 with a lack of chromosome 6, not a single backcross boll was formed when crossing with the monosomic interspecific hybrid F_1 .

The setting of full-fledged backcross F_1BC_2 hybrid seeds also differed significantly (from $8,43 \pm 2,16$ to $64,35 \pm 4,47\%$), where in nine variants a decrease in the percentage of set hybrid seeds was observed, and in four backcross F_1BC_2 variants (with lines Mo58, Mo59, Mo92 and Mo27) - an increase in this indicator compared to F_1BC_1 hybrids.

Thus, over the course of three hybrid generations, in eight studied variants (with the participation of lines Mo38, Mo60, Mo75, Mo34, Mo94, Mo48, Mo17, and telo21), there was a linear decrease in the set of hybrid seeds. Only one Mo27 line with a lack of chromosome 7 was characterized by an increase in this indicator, while the remaining four monosomic lines (Mo16, Mo58, Mo59, and Mo92) were distinguished by alternating crossbreeding indicators in different hybrid generations. In general, significant differences in the set of hybrid seeds in the studied F_1 , F_1BC_1 and F_1BC_2 variants were due, along with the hybridity of the material, to the low seed set in many initial monosomic lines with haplo-deficiency, as well as a small number of seeds per boll due to the formation of a large number of haplo-deficient gametes with shortages of individual chromosomes.

Comparative analysis of chromosome conjugation in hybrid monosomic F_1 obtained from crossings of 23 monosomic lines with the donor line Pima 3-79 of the species *G. barbadense* L. revealed normal modal chromosome conjugation with the formation of 25 bivalents and one univalent of different sizes in all studied PMCs. However, one F_1 monosomic hybrid plant from the Mo34xPima 3-79 family was characterized by the presence of additional univalents ($1,09 \pm 0,16$ on average per cell). In another monosomic hybrid plant F_1 from the Mo95xPima 3-79 family, one quadrivalent was formed in one PMC ($0,07 \pm 0,06$ on average per cell). Whereas the analysis of chromosome conjugation in F_1 hybrid monosomics obtained from crossings of two monotelodisome lines with the donor line Pima 3-79 of the species *G. barbadense* L. revealed the formation of 25 normal

closed bivalents and one heteromorphic bivalent. In backcross generations, haplo-deficient hybrid plants were not found in all F_1BC_1 hybrid families, since success in reproducing the monosomic state was highly dependent on the rate of reproduction in the hybrid progeny. In cotton, there are differences between the specific chromosomes of the cotton genome in terms of reproduction rates [14].

The study of chromosome conjugation carried out at the metaphase I stage of meiosis in hybrid monosomic F_1BC_1 obtained from crossings of 14 monosomic lines with interspecific monosomic hybrids F_1 (MoxPima 3-79) with substitution on chromosomes **2, 4, 6, 7, 12, 18, 21** and **22** found normal, modal chromosome conjugation with the formation of 25 bivalents and one univalent of different sizes in all studied PMCs, while in one monosomic hybrid plant in the F_1BC_1 (Mo75xF11042) variant, from one to three open bivalents ($0,33\pm 0,25$ on average per cell), along with normal closed bivalents ($24,67\pm 0,25$ on average per cell), which indicated incomplete conjugation of homologous chromosomes due to hybridity and possibly. The study of chromosome conjugation in two variants with monotelodisomic lines revealed the presence of heteromorphic bivalents, indicating the lack of a separate arm of chromosomes 6 and 11, (respectively).

Chromosome conjugation analysis performed at the metaphase I stage of meiosis in F_1BC_2 hybrid monosomic lines obtained from crossings of nine monosomic lines with F_1BC_1 interspecific monosomic hybrids (MoxPima 3-79) with substitution on chromosomes **4, 6, 7, 12, 18** and **22** revealed normal, modal conjugation of chromosomes with the formation of 25 bivalents and one univalent of different sizes in all studied PMCs, while in one monosomic hybrid plant in the F_1BC_2 (Mo17x F_1BC_1 110₁) variant, from one to three open bivalents were observed in individual cells, along with normal closed bivalents, which indicated incomplete conjugation of homologous chromosomes. The study of chromosome conjugation in the variant with the monotelodisomic line revealed the presence of heteromorphic bivalents, indicating the lack of a separate arm of chromosome **11**, along with the formation of a quadrivalent.

4 Conclusions

Some monosomic and monotelodisomic forms of *G. hirsutum* L. cotton are difficult to cross with the donor line Pima 3-79 of *G. barbadense* L. The indicators of crossability and setting of hybrid seeds in primary crosses are much higher than in backcrosses. However, crossability and backcross seed setting in F_1BC_1 is significantly lower than in F_1BC_2 . Significant differences in crossability in the studied hybrid variants F_1 , F_1BC_1 and F_1BC_2 are due to the interspecific features of the crossed species and the specificity of the lack of individual chromosomes in the tested monosomic cotton lines. Difficulties in combining the flowering period when backcrossing monosomic and monotelodisomics cotton lines with aneuploid hybrids of different generations are due to the reduced flowering rate of lines with a shortage of individual chromosomes or their arms. The rate of reproduction of hybrid plants with cotton haplo-deficiencies depends both on the specificity of the monosomic and on the number of plants in the hybrid offspring.

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