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Cytogenetic Collection of Uzbekistan

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<http://dx.doi.org/10.5772/58589>

1. Introduction

The genetic improvement of cotton demands the use of cytogenetic stocks for molecular mapping of QTLs and introgression of beneficial genes from wild and unadapted germplasms in Upland cotton. Development of the collection of cotton cytogenetic lines with translocations and chromosome deficiencies are necessary to fulfill these goals. During long-term studies the series of translocations and monosome stocks were developed in the USA, which provide chromosome identification and localization of marker genes on chromosomes. As the result, totally, 63 translocations were accumulated over a period of more than 20 years in USA [1-2]. The 62 heterozygous translocations were transferred to homozygous state and identified. Twenty reciprocal translocations of the cytogenetic tester set of *G. hirsutum* were selected and cytologically characterized [3,4]. This tester set of the translocations marks 25 of the 26 chromosomes of *G. hirsutum*, with chromosome 26 being identified by elimination.

Cultivated allotetraploid cotton, *G. hirsutum* ($2n=52$), is tolerant to the loss of individual chromosomes or their arms. During long-term investigations a big number of monosomic plants of different origin were isolated in the USA [5-9]. The majority of monosomes were found for chromosomes 2, 4 and 6 of the A-genome [10]. Unfortunately, the complete series of 26 monosomic lines in cotton have not been recovered yet. The monosomes for 15 of the 26 nonhomologous chromosomes of *G. hirsutum* were identified [2]. Therefore the development of one or more deficiencies which would involve a part and/or all of these chromosomes has been high priority [11]. Use of the new molecular cytogenetic methods – meiotic fluorescence in situ hybridization (FISH) was identified a new cotton monosome of chromosome 23 [12]. Recently, another new monosome for chromosome 21 in cotton was reported [13]. During the last years the monosomic stocks were used for chromosome assignment genetic and molecular markers to specific chromosomes [14-21].

Use of F₁ hypoaneuploid hybrids resulting from the crosses of *G. hirsutum* aneuploids (2n-1 or 2n-1/2) and *G. barbadense* L. species (2n) in molecular-genetic analyses has facilitated the localization of different molecular markers on specific cotton chromosomes [22-25]. However, some loci were not assigned using the aneuploids due to the lack of a full set of cotton aneuploids [e.g. 21, 25-27]. In the last decade chromosome-deficient stocks of *G. hirsutum* have been used for the development of chromosome substitution lines for *G. barbadense*, *G. tomentosum* and *G. mustelinum* chromosomes or chromosomes segment(s) [28-29].

In Uzbekistan the investigations to induce chromosome aberrations and to develop new translocation and chromosome deficient stocks were conducted during more than 30 years [30-43]. As the result 94 primary and 22 tertiary monosomics, 20 monotelodisomics, 4 monoisodisomics, 235 reciprocal translocations as heterozygotes, 33 homozygous translocation stocks, 4 haploids and 31 desynaptic plants were discovered (Table 1). Here we report cytogenetic and morphological characteristics of the new cotton translocation and monosomic lines. We also report the results of identification of some of the lines by means translocation test.

Origin	Number of plants							
	Translocation as heterozygote	Translocation as homozygote	Primary monosomic	Tertiary monosomic	Monotelo- disomic	Monoiso- disomic	Haploid	Desy- naptic
Combine treatment	23	13						
Neutron irradiation	72	14	10	9	1		1	11
Irradiation of pollen	140	6	65	13	10	3	2	20
Desynapsis			17					
Translocation			2					
Progeny of monosomic					9	1	1	
Totals	235	33	94	22	20	4	4	31

Table 1. The origin of the different aberrations in cotton *G.hirsutum* L.

2. Materials and methods

Inbred cotton lines L-458, L-461, L-500 and L-501 (*Gossypium hirsutum* L.) from the Genetic Collection of the National University of Uzbekistan were used for producing cytogenetic stock series. Three types of radiations were applied – combined treatment of colchicine and gamma

rays (A) of the seeds, irradiation of seeds by thermal neutrons (B) and pollen gamma irradiation rays (C). The seeds of the hybrids F_0 (L-500 x L-461) were treated with 0.1% aqueous colchicines solution for 2 h, washed with water and irradiated with CO^{60} gamma-radiation at 50 and 100 Gy. The seeds used in this study were of the M_0 generation.

The irradiation of pollen by gamma rays was carried out in Institute of Silk research. The flowers of cotton line L-458 were emasculated a day before flowering and enclosed in parchment bags to prevent accidental crosspollination and in flowering stage the flowers with mature pollen were collected and irradiated with 10, 15, 20 and 25 Gy gamma radiation (Co^{60}). The irradiated pollen used to pollinate the emasculated flowers.

The neutron irradiation of cotton line L-458 seeds was carried out at the biological channel of the WW-SM reactor using doses of 15, 25, 27 and 35 Gy (Institute of Nuclear Physics of the Academy Sciences of the Republik of Uzbekistan, Tashkent). The seeds of M_0 generation were grown in same field condition. The M_1 plants were studied and used to raised the M_2 generation. The M_2 plants were grown in a field and investigated for chromosomal aberrations using cytological techniques.

The primary monosomics from our collection were numbered from Mo1 to Mo94 in the order of their detection. Transmission of the monosomics was studied in selfing and outcrossing progenies of monosomic plants both in the greenhouse and in the field. Translocation and monosomic lines were identified. Hybrids between translocation and monosomic lines were analyzed to identify $2n$ and $2n-1$ translocation heterozygotes, respectively. Meiotic chromosomes were studied using the standard acetocarmine-squash technique [30].

3. Cytological analysis

The chromosome pairing at metaphase I of meiosis was studied. The calyx and corolla were removed and floral buds were fixed overnight in 96% alcohol and acetic acid (7:3). Buds were kept at room temperature for 3 days, immersed in fresh fixative and stored in a refrigerator. They were examined for meiotic associations in the pollen mother cells (PMCs) using iron acetocarmine squash technique [30]. The analysis of chromosomal changes was carried out on the basis of M I associations at the first meiosis. The development of PMCs was examined at the tetrad stage for each plant. The meiotic index was calculated as the percentage of tetrads whereas pollen fertility was estimated by acetocarmine staining.

4. Results

4.1. Reciprocal translocations

Reciprocal translocations were formed as segmental interchanges among two or more nonhomologous chromosomes. The exposure of seeds to three types of irradiation has resulted in the induction of translocations in 235 cotton plants [31, 32, 34, 37]. After different treatment

of irradiation translocation plants were obtained in the M_1 , M_2 and M_3 generations (Tables 2). They included exchanges between two different pairs chromosomes, translocations among three pair chromosomes and complex exchanges. Three types of the irradiation treatment were characterized by differences of the frequency and spectrum of the translocations. The treatment of seeds with combined colchicines and gamma-rays resulted 25 disomic plants with two or more translocations in the M_1 generation, with two, or even four multivalent configurations per PMCs in different plants. The unaffected plants from M_2 generation, a further 22 disomic plant with a number multivalents were detected. Progeny from different branches and bolls of one and the same parents was kept separate. Some of the parents were chimeras and as it was expected and subsequently confirmed, that different bolls from one and the same parent would give different result. From the meiosis, M_3 progenies were only recovered in which there was just one multivalent per PMC. Therefore, 23 translocations obtained in the M_3 generation were listed in the Table 1. In comparison to the single irradiation the combined irradiation treatment was most effective for producing the highest number of single as well as complex translocations in a number of meiocytes.

Treatment	Dose of irradiation (Gy)	Number of translocation heterozygotes			Translocation lines
		M_1	M_2	M_3	
Combined treatment of seeds by colchicine and gamma-rays	50	19	19	19	Tr1-Tr11, Tr25, Tr26
	100	6	3	4	0
Subtotal		25	22	23	13
Irradiation of seeds by thermal neutrons	1,3	1	0	0	0
	15	8	11	-	Tr17-Tr19, Tr22, Tr27, Tr30
	25	9	7	-	Tr20, Tr21
	27	7	8	10	Tr15, Tr16, Tr28, Tr31, Tr33
	35	6	5	-	Tr29
	Subtotal	31	31	10	14
Irradiation of pollen by gamma-rays	10	3	9	13	0
	15	9	13	7	Tr12
	20	16	20	12	Tr13, Tr14
	25	25	10	3	Tr23, Tr24, Tr32
	Subtotal	53	52	35	6

Table 2. The origin of the reciprocal translocations in cotton *G. hirsutum* L.

Irradiation of seeds by thermal neutrons induced 31 translocations in the M_1 , 31 plants with interchanges in the M_2 and 10 - in M_3 generation (Table 2). Only four plants were recorded

with complex translocations per PMC in the M_1 generation (Figure 1). Other translocations involved to two nonhomologous chromosomes (Figure 2) and four – of three chromosomes. Moreover, the concurrent appearance of the two chromosomal aberrations causing and inducing chromosomal deficiencies and rearrangements, was also detected. The highest number of the chromosome changes occurred from the thermal neutron and unique chromosomal aberrations such as complex of the interchanges on PMS, multiple translocations involving up to three nonhomological chromosomes.

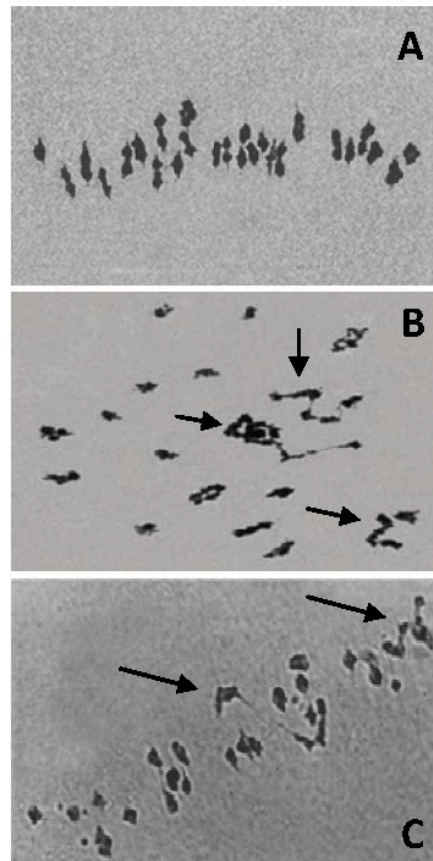


Figure 1. Meiotic configurations in translocation heterozygotes in cotton *G. hirsutum* obtained after irradiation of the seeds by thermal neutrons in M_1 . (A) Meiotic metaphase I cell showing 26 bivalents in control plant. (B) Meiotic metaphase I cell showing 19 bivalents and 2 quadrivalents and 1 hexavalent in plant 364/10. (C) Meiotic metaphase I cell showing 21 bivalents and 1 quadrivalent and 1 hexavalent in plant 364/10. The arrows point to the quadrivalents and hexavalent. Note that the background of figures was cleaned using Adobe Photoshop CSS extended version 12

Moreover, such rare mutations induced have not been observed earlier in experiments with other treatments. Such rare mutations were cluster fruiting habit for translocation (Tr18-Figure 10 C, F), reduced stigma (Mo62-Figure 18 C), cytoplasmic mutation virescent simultaneous with translocation (Tr28), unique desinaptic plant, pollen semisterility in homozygous stock (Tr21). It is also notable that one of the translocation plant 1475/30₄ had also a clear phenotypic character with cytoplasmic mutation of yellow-green color of the leaf. This finding indicates the simultaneous appearance of chlorophyll deficiency and the chromosome translocation in

a single plant. These specific mutations were not frequent, but they were specific for the thermal neutron irradiation and very important for cotton genetics as new genetic markers.

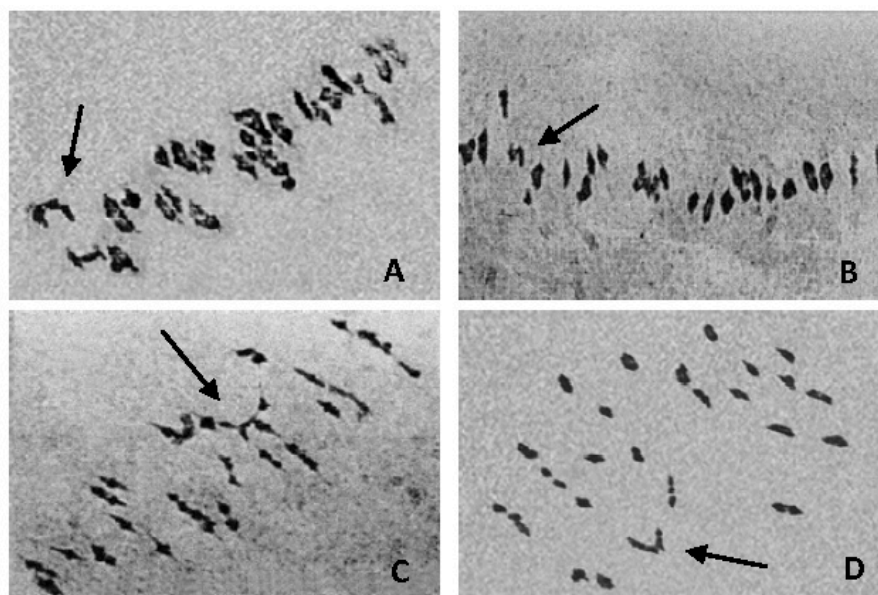


Figure 2. Meiotic configurations in translocation heterozygotes in cotton *G. hirsutum* obtained after irradiation of the seeds by thermal neutrons in M_3 . Meiotic metaphase I cells showing 24 bivalents and 1 quadrivalent: in plant 1475/7₁-13 (A and B); (C) 1474/15₄-6; (D) 361/4-b1₃. The arrows point to the quadrivalents. Note that the background of figures was cleaned using Adobe Photoshop CSS extended version 12.

A similar analysis of chromosome aberrations showed that gamma-irradiation of pollen resulted in 53 translocations in the M_1 out of 331 studied plants, 52 plants with interchanges in the M_2 out of 348 Studied plants and 35 - in the M_3 generation out of 211 studied plants (Table 2). All translocations were involving of two nonhomologous chromosomes formed quadrivalent associations (Figure 3). Three translocations were involving of three chromosomes formed hexavalent associations. There were no PMCs with more than one multivalent among translocation plants after pollen irradiation. The greatest number of translocation plants with medium and high frequency (58.14%) was found in experiments with irradiation at 20 and 25 Gy. In comparison to the other irradiation the gamma-irradiation of pollen was the most effective for producing more different deficiencies.

Comparison of the mean frequency of multivalent per cell between translocations obtained in the M_1 , M_2 and M_3 generations after irradiation suggests that the highest number (26,42%) of interchanges with a high average number of multivalent at the MI of meiosis occurred in the M_1 generation. M_2 and M_3 progenies had more normal karyotypes than that seen in the M_1 plants and translocations with a high frequency of multivalent were uncommon in next generations. Those translocations, integrated into this collection from homozygous translocation lines.

Among 235 translocations, 224 involved two chromosomes but only 11 involved three nonhomologous chromosomes. Different types of multivalent configurations were found with

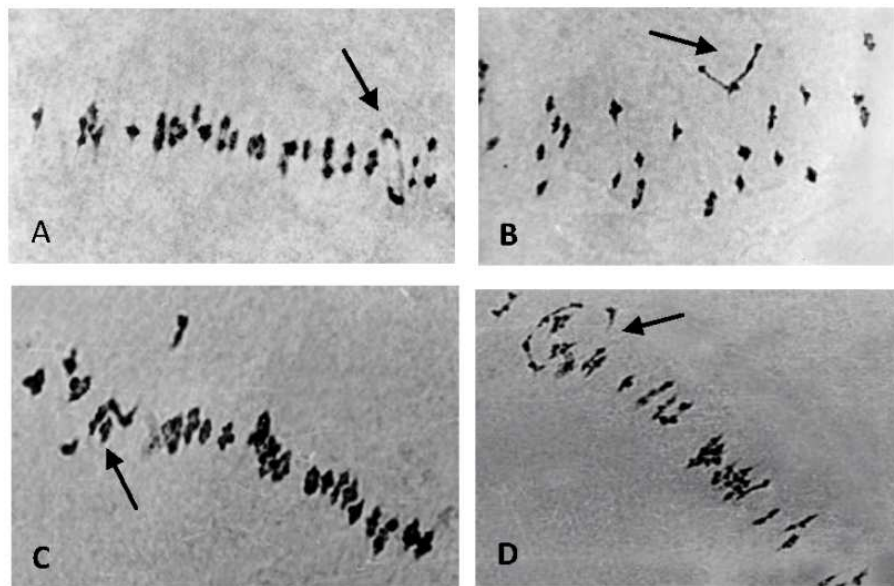


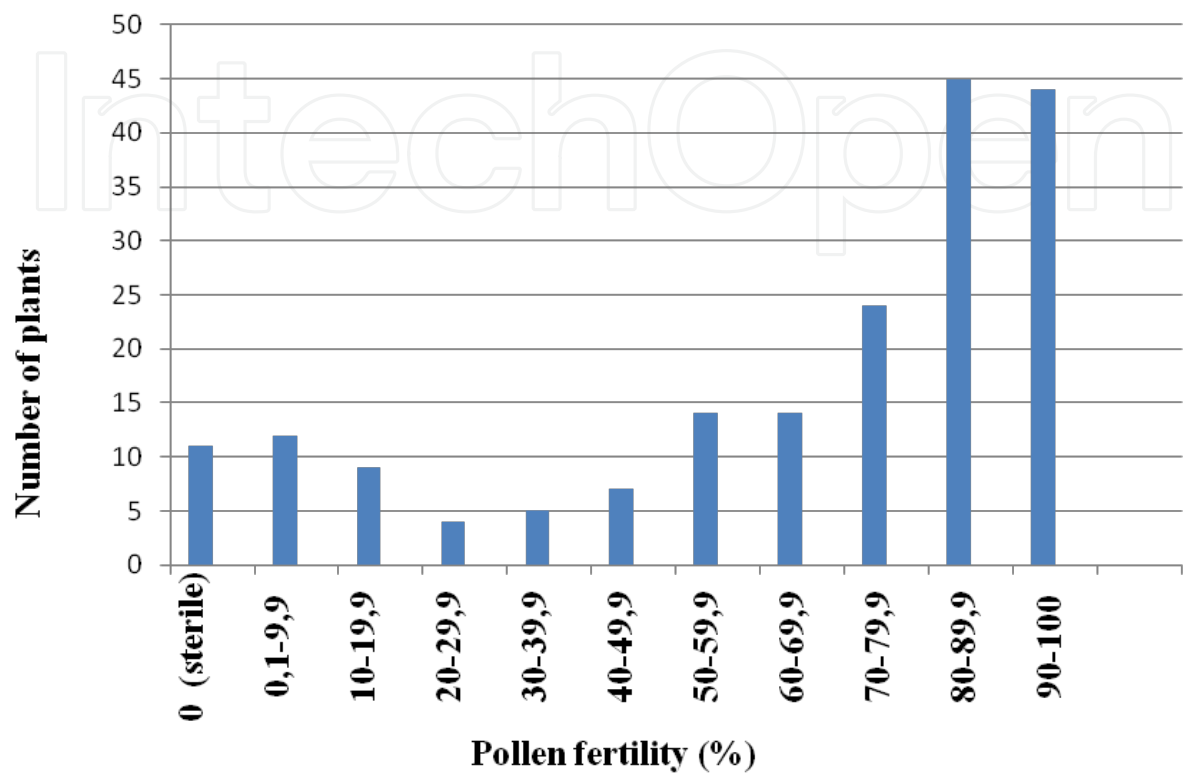
Figure 3. Meiotic configurations in translocation heterozygotes in cotton *G. hirsutum* obtained after pollen irradiation in M_2 and M_3 . Meiotic metaphase I cells showing 24 bivalents and 1 quadrivalent in plant (A) 1069/10₁; (B) 1568/21₁₀; (C) 188/4₄-3; (D) 187/3₁₀-12. The arrows point to the quadrivalents. Note that the background of figures was cleaned using Adobe Photoshop CSS extended version 12.

alternate and adjacent orientations. The translocations were characterized by different frequencies of the multivalents at the MI of meiosis. Translocations with the large translocated chromosome segments are of greatest interest for tagging cotton chromosomes and developing new homozygous translocation lines. Electron microscopy of synaptonemal complexes at the pachytene stage revealed more cells with heterozygous chromosomal rearrangements than light microscopy at meiotic MI by a factor of 1.8 [44]. Those results indicate that the different frequencies of multivalents in heterozygotes for translocations at MI result from partial desynapsis and segregation of translocation multivalents into two “heteromorph” bivalents, which cannot be distinguished from normal bivalents at meiotic MI by light microscopy.

The analyses of tetrads were carried out in 201 translocations. Most of the translocations (76.41%) exhibited a high meiotic index (90-100%) when compared with the control (94.52±0.26). However, 20.51% translocations were characterized by a reduction in meiotic index (to 80.0%) and an increase in the percentage of tetrads with micronuclei when compared with the control (1.29±0.13%). Six translocations were characterized with a low meiotic index (from 52.15±1.99 to 79.37±1.32%) and number of abnormal tetrads containing micronuclei (from 16.53±1.48 to 3.66±0.53%).

The distribution of pollen fertility for 189 translocations is summarized in Figure 4. Pollen fertility was estimated by acetocarmine staining. Although the acetocarmine-based pollen fertility considered relatively insensitive method, it is widely used for preliminary screening of pollen quality in plants. The pollen fertility of translocations varied significantly from high fertility (70-100%) for 113 translocations, to semi-sterility (40-69.9%) for 35 translocations, and to low fertility (to 39.9%) for 23 translocations when compared with the control (96.99±0.44%).

In 19 translocations, pollen fertility varied between flowers of one and the same plant and 11 translocations were characterized by pollen sterility. On the whole, the high frequency of abortive pollen grains in flowers was typical for 48.5% of the translocations studied.



Note: the remaining 19 translocations were not included in the histogram due to their varied pollen fertility level in different flowers and 4 plants were complex interchanges.

Figure 4. Percentage distribution of pollen fertility for 189 reciprocal translocations in cotton

This broad variability of pollen fertility in the plants with interchromosomal exchanges hampers using this trait as a marker of heterozygosity for exchanges in cotton, in contrast to species of *Pisum*, *Zea*, *Sorghum*, and *Petunia*. Heterozygosity for translocations in these species is always accompanied by half-sterile pollen because of equal probabilities of ring-shaped and zigzag-shaped quadrivalent orientation. In addition, the detection of complete male and female sterility in some cotton translocants suggested that exactly the translocations were responsible for their sterility so far as these translocation plants did not produce any seed sets from self-pollination and intercrossing. Apparently, short translocated segments involved vital chromosome domains, whose rearrangements induced to abortive gametes.

Different techniques have been employed for isolating plants homozygous for the translocations [45]. Common methods have been used in maize and barley [46]. To identify homozygous, self the plants with normal pollen crossed to a standard normal line. If the normal being tested were homozygous for the translocation, all the F₁ hybrid plants should be partially sterile. A technique for quick isolation of translocation homozygotes that not require of the

analysis pollen fertility into account was also worked out. This character has been varied in the cotton translocation heterozygotes from high up to pollen sterility and cannot be used the marker characteristic. Such techniques allowed us to isolate the number of different translocation homozygotes in progeny of one and the same parent containing two or more interchanges per PMC.

Translocations have been confirmed as homozygous after cytogenetic studies from self-pollination progenies of the heterozygotes according to the scheme (Figure 5). Heterozygotes were selfed and progeny from each plant was examined at the metaphase I of meiosis. The plant that exhibited normal pairing were backcrossed to the control line L-458 in order to identify the plants homozygous for the translocations and the F_1BC_1 progeny was examined at the metaphase I of meiosis for the presence of multivalents. F_1BC_1 plants with multivalents pointed out homozygous for the translocation under consideration. As a result 33 new homozygous translocation stocks were isolated among those, 13 new translocation stocks of cotton (Tr1-Tr11, Tr25 and Tr26) were obtained in the combined treatment of seeds with colchicine and γ -rays hybrid progeny L-500 \times L-461, one stock (Tr12) – in the pollen γ -irradiation hybrid progeny L-461 \times L-501, while the others – from irradiation of seeds by thermal neutrons highly inbred line L-458.

In the progeny of the translocation heterozygotes, the deviations were found from the 1:2:3 ratio with a deficit of different types of plants. The latter can be explained by time limitation of examining plants or low viability by some types of progeny. So, it was not possible to establish one translocation (1020₁-9) in the homozygous condition because only heterozygotes and normal plants detected in progeny. Probably, it can be attributed to localization of the breakpoints in the region of the chromosome, which cannot be reconstructed. As it was discussed earlier for 28 of the 34 translocation lines in barley, homozygous plants were available, although one translocation – C 951 was not identified in the homozygous condition [47].

Translocation lines from our collection were numbered from Tr1 to Tr33 in the order of their detection. Thirty one translocations were simple reciprocal interchanges, involving only two nonhomologous chromosomes, whereas the two remaining (Tr2 and Tr20) were interchanges involving three non-homologous chromosomes. Translocation lines were characterized by normal pairing at metaphase I of meiosis with 26 bivalents and high meiotic index (from 90.73 ± 0.33 for Tr24 to 98.05 ± 0.19 for Tr22). The pollen fertility was also high (from $90.06 \pm 0.77\%$ for Tr15 to 98.28 ± 0.18 for Tr18) with the exception of Tr21 that was distinguished with a essential decrease in pollen fertility (to $66.01 \pm 1.28\%$). For the first time such semisterility of the pollen was detected in the cotton plants homozygous for the translocation. Such differences can be explained by heterogeneous translocations.

Subgenome assignment of the translocation was carried out with using hybrid DD-subgenome (F_1 *G. thurberi* \times *G. raimondii*). Three types of modal configurations are expected at metaphase I of meiosis in the triploid hybrids [48]. Translocations being involving two A-subgenome chromosomes will have not A homoeologues in the triploid hybrid and showed modal chromosome configurations – 13 (DD) bivalents and 13 (A) univalents; translocations being involving two DD-subgenome chromosomes – 11 (DD) bivalents and 13 (A) univalents and

one (DDDD) quadrivalent; translocations being involving AD-subgenome chromosomes – 12 (DD) bivalents and 12 (A) univalents and one (ADD) trivalent. Tests involved many translocation lines but results were obtained for 5 lines only.

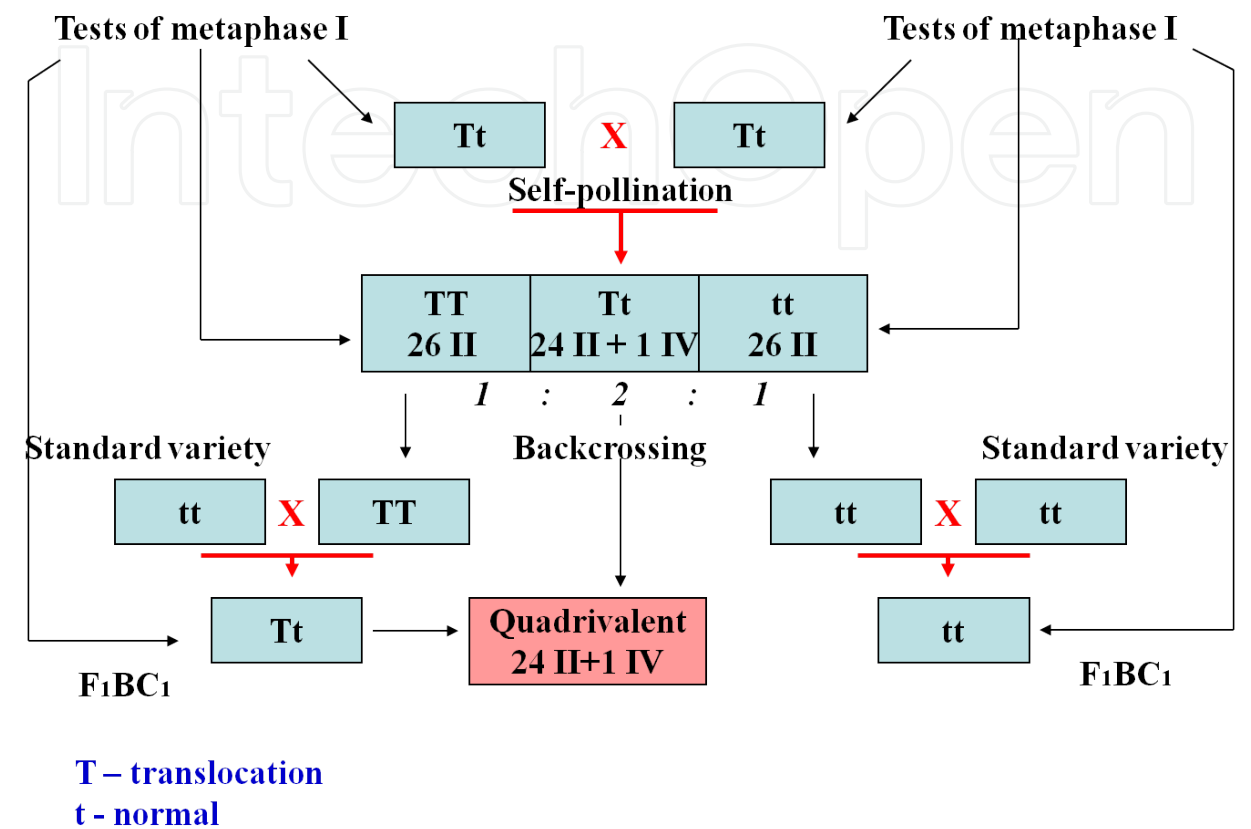


Figure 5. Scheme of the technique used for the isolation of translocation homozygous lines (further explanations noted in the text)

Translocation lines Tr1, Tr7, Tr8 and Tr16 had AA-subgenome location translocated chromosomes because their triploid hybrids shown modal configurations 13 DD-bivalents and 13-A univalents (Figure 6). Translocation line Tr2 is interchange which involves three non-homologous chromosomes. Their triploid hybrid characterized 11 univalents and 12 bivalents and one quadrivalent that pointed out on two A-subgenome and one D-subgenome location chromosomes.

Identification of translocation homozygotes from our collection was carried out using double translocation heterozygotes obtained after intercrossing translocation plants [1]. Modal chromosome configurations at metaphase I of meiosis – 21 bivalents (II)+1 quadrivalent (IV)+1 hexavalent (VI) and 22 bivalents (II)+2 quadrivalents (IV) showed the involvement the different chromosomes, modal chromosome configurations – 22 bivalents (II)+1 oktaivalent (VIII) and 23 bivalents (II)+1 hexavalent (VI) indicated the chromosome in common and modal chromosome configurations – 26 bivalents (II) indicated the involvement of the same arms of the two homological chromosomes. It is important to note that

translocation lines Tr2 and Tr20 showed multivalents in hybrids because of involving the translocations among three non-homologous chromosomes. With respect to the crosses between translocation lines Tr7, Tr8 and Tr9 in which the M_1 parent plant was formed of several quadrivalents in the same meiocyte, three types were present in homozygote giving rise to the three translocation lines. The data indicated that Tr7 and Tr8 involved the same two non-homologous chromosomes, but Tr7 and Tr8 on the one hand and Tr9 on the other hand are different chromosomes.

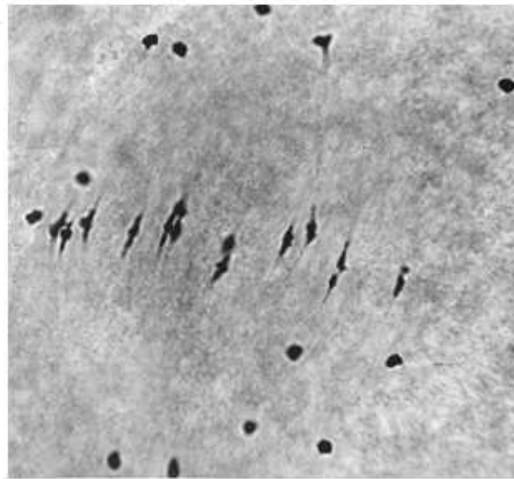


Figure 6. Meiotic configuration in hybrid from crossing the translocation line Tr 16 and hybrid F_1 (*G. thurberi* x *G. raimondii*). Meiotic metaphase I cells showing 13 univalents and 13 bivalents. Note that the background of figures was cleaned using Adobe Photoshop CSS extended version 12.

The differences were found between translocation stocks both in the number of hybrids and the number of common chromosomes in the translocations in our investigation. Thus, the translocation lines Tr7, Tr8, Tr14, Tr16 and Tr27 showed more common chromosomes, whereas other lines-Tr1, Tr18, Tr20, Tr21, Tr23 and Tr26 showed uncommon ones. From these data it is observed that chromosomes in the translocations Tr7, Tr8, Tr14, Tr16 and Tr27 were more frequently involved in chromosome translocations, but chromosomes from the translocations lines-Tr1, Tr18, Tr20, Tr21, Tr23 and Tr26 were less frequently involved in translocations (Figure 7). On the base of rare occurrence the chromosomes in common in the hybrid involved Tr1 and Tr20 translocation stocks (two and one, respectively) it was shown that these stocks have translocated chromosomes that were rare involving into interchanges. Thus, when Tr1 stock was crossed with Tr2 and Tr20 the ring consisting of 8 chromosomes was observed in both hybrids showing that Tr1 stock has interchanged chromosomes in common with Tr2 and Tr20 stocks. However, the crosses between Tr2 and Tr20 stock detected two rings consisting of six chromosomes that pointed out the involvement unique chromosome pair in Tr1 and Tr20. Preliminary chromosome numeration in the interchanges pointed out the involvement about 50% chromosome set into reciprocal translocations in 27 studied translocation stock from our collection.

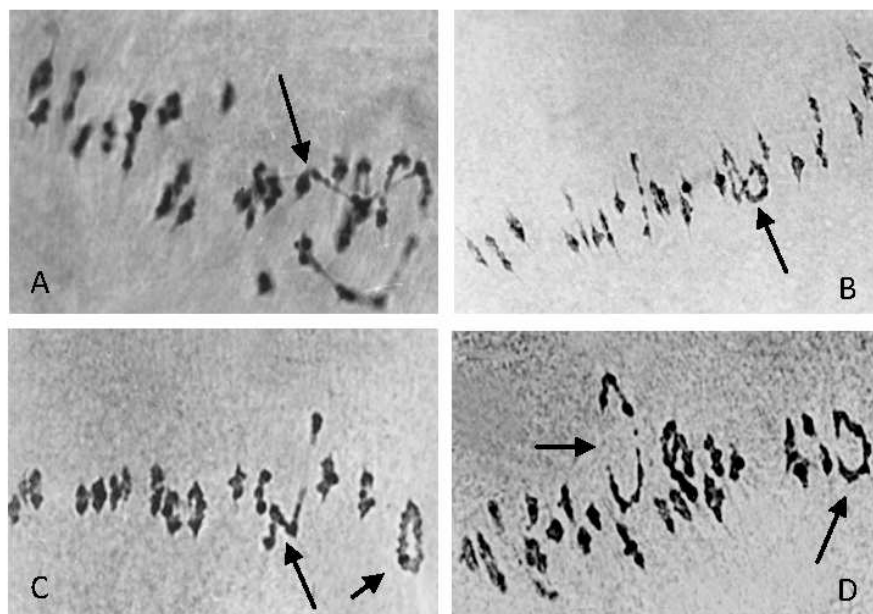


Figure 7. "Critical configurations" of the chromosomes at the meiotic metaphase I cells in cotton F_1 plants from crosses the different translocation lines: (A) Tr2 x Tr16 (22 II+1 VIII); (B) Tr8 x Tr6 (23 II+1 VI); (C) Tr1 x Tr23 (22 II+2 IV); (D) Tr15 x Tr10 (22 II+2 IV). The arrows point to the quadrivalents, hexavalent and oktavalent. Note that the background of figures was cleaned using Adobe Photoshop CSS extended version 12.

Moreover, translocation lines are different in morphological characters. 13 new translocation stocks of cotton *G. hirsutum* (Tr1-Tr11, Tr25 and Tr26) were obtained from the hybrid progeny (L-500 x L-461) after combine treatment, where two parent lines (L-500 and L-461) distinguished by leaf shape. 6 translocation lines (Tr1, Tr2, Tr4, Tr6, Tr9 and Tr26) have leaves palmate, such as 7 lines (Tr3, Tr5, Tr7, Tr8, Tr10, Tr11 and Tr25) – lanceolate (super okra) (Fig. 8). Moreover, translocation line Tr10 be showed dense growth and bract type "frego" (Fig. 8 and 10 B). Line Tr12 have compact grow, small round leaves and small spherical boll (Figure 8).

The other 19 translocation lines were obtained by the irradiation of the seeds line L-458 of the thermal neutrons (Figure 9).

The line Tr18 was characterized by compact grow, a dense stem pubescence, cluster flowers and bolls and absent of stigma protrusion over the staminate column (Fig.10 A, C, D, F). The line Tr23 differed by dense stem of pubescence. Tr28 line demonstrated yellow color of the leaf resulted from simultaneous cytoplasmic mutation type virescent. Moreover, Tr28 line showed difference in absence of external nectarines (Fig 10 E). Thus, it was shown that chromosome translocations had a specific influence in plant morphology and that some of translocations distinguished unique marker characters (Figure 10).

Final conclusions cannot be drawn without further cytogenetical studies to identify the chromosomes involved in the translocations. It is quite evident that translocation stocks of our collection may be used for marking cotton chromosome, for the identification of the monosomes and other chromosomal aberrations.



Figure 8. Some examples of morphology of cotton translocation lines (Tr10, Tr11, Tr25, Tr26, Tr12) compared to original parental lines (L-500, L-461, L-501).

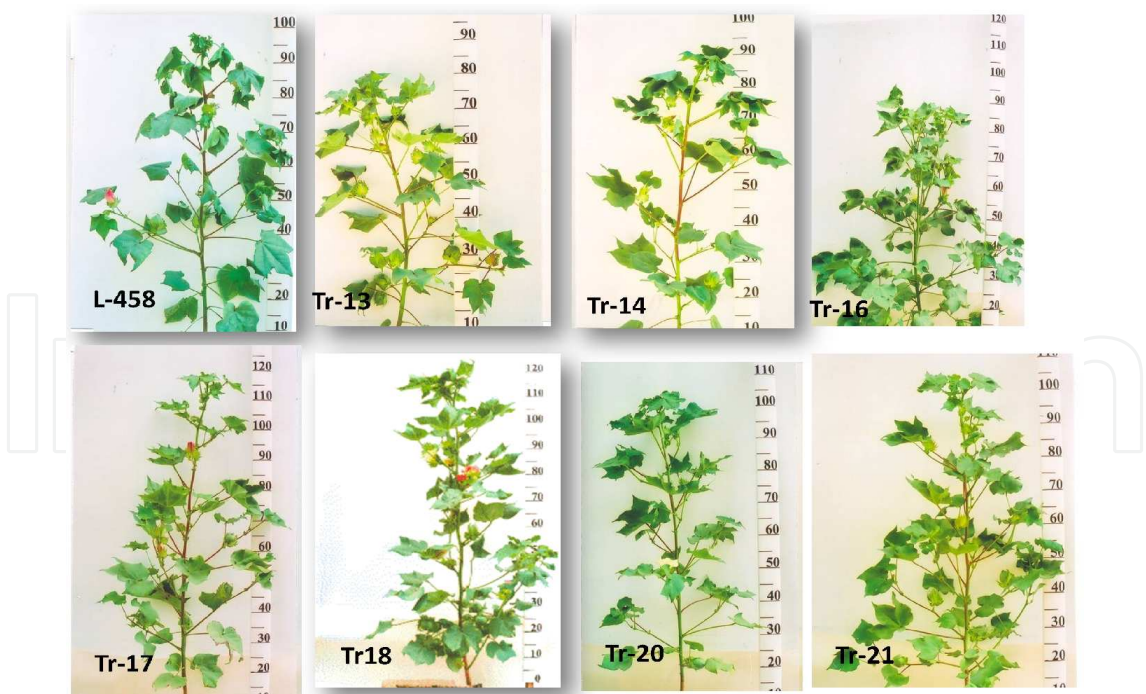


Figure 9. Some examples of morphology of cotton translocation lines (Tr13, Tr14, Tr16, Tr17, Tr18, Tr20, Tr21) compared to original parental line (L-458).



Figure 10. Some unique morphologic characters of the translocation lines: (A) a dense stem pubescence of the translocation lines Tr18 (1) and Tr23 (3) in comparison with parental line L-458 (2); (B) bract type "frego" of the translocation line Tr10; (C) cluster flowers in translocation line Tr18; (D) absent of stigma protrusion over the staminate column in translocation line Tr18 (2) in comparison with parental line L-458 (1); (E) absence of external nectarines in translocation line Tr28 (1) in comparison with parental line L-458 (2); (F) cluster bolls in translocation line Tr18.

5. Primary monosomics of the *G. hirsutum* L.

The cultivated allotetraploid cotton, *G. hirsutum* ($2n=52$), was tolerant to the loss of individual chromosomes or their arms. One of chromosome missing in monosomic cotton plant which has only 51 chromosomes. Meiotic metaphase I analysis of cotton primary monosomics are revealed modal chromosome pairing with 25 bivalents and univalent. Between 1987 and 2010, we developed a total of 94 *G. hirsutum* primary monosomics from the common genetic background of the highly inbred line L-458 after irradiation of seeds by thermal neutrons or pollen gamma-irradiation directly in M_1 , M_2 and M_3 generations. Most of them (75 of 94) observed from the two irradiation types directly in M_1 , M_2 and M_3 generations. The remaining 19 monosomic plants resulted from chromosome aberrant progenies (desynapsis and interchanges). Most of the primary monosomics (34) were induced in the M_1 generation as a result of pollen γ -irradiation by doses of 10, 15, 20 and 25 Gy (Table 3).

Treatment	Dose of irradiation (Gy)	Number of primary monosomics			Monosomic lines
		M ₁	M ₂	M ₃	
Irradiation of seeds by thermal neutrons	1,3	0	0	0	-
	15	3	1	0	Mo58, Mo59, Mo60, Mo74
	25	0	1	0	-
	27	0	1	2	Mo1
	35	1	2	0	Mo56, Mo62
Total		4	5	2	7
Irradiation of pollen by gamma-rays	10	5	4	3	Mo10, Mo39, Mo40, Mo41, Mo50, Mo81, Mo82
	15	4	9	1	Mo3, Mo31, Mo53, Mo78
	20	11	8	4	Mo4, Mo7, Mo11, Mo22, Mo27, Mo28, Mo34, Mo35, Mo36, Mo66, Mo75, Mo90
	25	14	3	0	Mo9, Mo13, Mo15, Mo16, Mo17, Mo19, Mo38, Mo46, Mo48, Mo76, Mo77
Totals		34	24	7	34

Table 3. The origin of the cotton primary monosomics *G. hirsutum* L.

Seven of the monosomic plants had simultaneously independent chromosome interchanges so far as these shown both quadrivalent and univalent in MI meiosis. More than 70% of M₁ primary monosomics (25 of 34), were induced by high doses of pollen irradiation (20 – 25 Gy) (Table 3). The number of monosomics detected declined in subsequent generations (24 and 7, respectively), and one M₃ monosomic (Mo54) also displayed heterozygous translocation (Figure 11). A specific feature of the pollination with irradiated pollen of cotton was a lot of genomic mutations such as chromosome deficiencies, chromosome arm deficiencies (22.51%) in comparison with the neutron irradiation (16.85%). The latter resulted from elimination of whole chromosomes, chromosome arms, or even the complete paternal genome and yielded monosomic, monotelodisomic, and haploid plants.

Similar analysis of cotton plants from seed irradiation with thermal neutrons at doses of 15, 25, 27 and 35 Gy revealed fewer primary monosomics. After irradiation only 11 plants from three generations out of 335 studied plants were detected, moreover four of them were also interchanged heterozygotes. In M₁ generation there were only 4 chromosome deficient plants, 3 of them from the dose of 15 Gy and one from the 35 Gy. Similarly 5 monosomic plants were isolated in M₂ generation in all 4 doses, and two monosomic plants were identified from M₃ (Figure 12).

An addition to traditional radiation-induced cotton monosomics, we used the desynaptic effect which have been found to be a useful source of aneuploidy in other crops. Although desynaptic plants in different crops are usually sterile or show extremely low fertility, the desynaptic

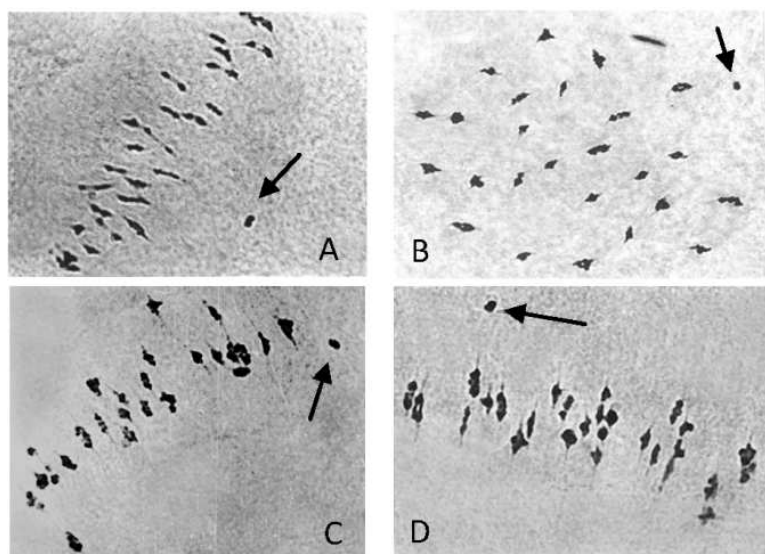


Figure 11. Meiotic configurations in primary monosomics in cotton *G. hirsutum* obtained after pollen irradiation in M_1 and M_2 . Meiotic metaphase I cells showing 25 bivalents and 1 univalent in plant (A) 1596/5; (B) 161/1; (C) 199/4₂; (D) 186/12₅.. Note that the background of figures was cleaned using Adobe Photoshop CSS extended version 12.

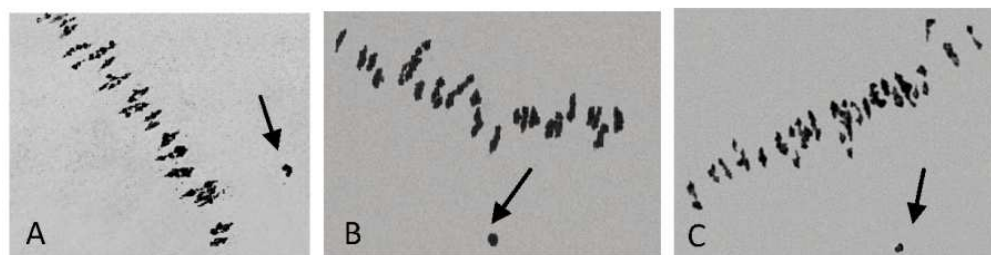


Figure 12. Meiotic configurations in primary monosomics in cotton *G. hirsutum* obtained irradiation of the seeds by thermal neutrons in M_1 , M_2 and M_3 generations. Meiotic metaphase I cells showing 25 bivalents and 1 univalent in plant (A) 1474/28₁₀₋₁; (B) 359/9; (C) 368/4₁. The arrows point to the univalents. Note that the background of figures was cleaned using Adobe Photoshop CSS extended version 12.

cotton plant 1063/6₃-13 had semisterile pollen due to different number of unpaired univalents (from 2 to 28) in different PMCs. Desynapsis level was estimated as intermediate. Other six desynaptic plants were characterized with weak desynapsis level and formed from 2 to 14 univalents. As a result, 17 primary monosomics were isolated from the progenies of 7 desynaptic plants and one unexamined plant from the desynaptic plant progeny (Table 4).

All the initial desynaptic plants differed by their number of unpaired chromosomes (from 2 to 28 univalents). Disruptions in unpaired chromosome disjunction led to a random univalent distribution between the cell division poles, forming numerous tetrads with micronuclei (to $13.42 \pm 0.87\%$), lowering of meiotic index (to $75.07 \pm 1.11\%$ in 356/8 desynaptic plant), and pollen fertility reduction to semisterility ($61.35 \pm 2.43\%$). Meiotic index is a normal tetrad percentage and an indicator of meiotic stability, which proposed by Love [49] for evaluation of meiosis in

wheat. We observed that pollen fertility was varied among the flowers on the same plant (from 2.61±1.27% to 91.81±1.18% in 179/2 desynaptic plant; Table 4).

Material	Mo	Chromosome number	Total number of cells	Chromosome associations			Pollen fertility	
				Number of univalents	Frequency of chromosome associations (in average per cell)		Total number of pollen grains	Fertility, (%)
					univalents	bivalents		
1609/6 ₆ -DPP		52	60	2-12	4.77±0.48	23.62±0.24	656	70.12±1.79
1609/6 ₆ -22	Mo55	51	22	1-3	1.45±0.18	24.77±0.09	466	93.99±1.10
1609/6 ₆ -4	Mo69	51	33	1	1.00±0.00	25.00±0.00	499	98.27±0.58
1063/6 ₃ -13-DPP		52	42	2-28	14.33±0.9	18.83±0.45	405	65.68±2.36
1063/6 ₃ -13 ₃	Mo70	51	25	1-3	1.08±0.08	24.96±0.04	639	95.15±0.85
1063/6 ₃ -13 ₄	Mo71	51	24	1	1.00±0.00	25.00±0.00	352	96.02±1.04
1063/6 ₃ -13 ₅	Mo72	51	21	1	1.00±0.00	25.00±0.00	632	96.87±0.69
1063/6 ₃ -13 ₆	Mo73	51	30	1-3	1.47±0.15	24.77±0.08	612	98.53±0.49
1570/14 ₉ -3-DPP		52	42	2-8	2.43±0.31	24.79±0.15	440	94.09±1.12
1570/14 ₉ -3 ₁₈	Mo78	51	18	1	1.00±0.00	25.00±0.00	-	-
1570/14 ₉ -13-DPP		52	15	2-6	2.67±0.46	24.67±0.23	467	95.29±0.98
1570/14 ₉ -13 ₇	Mo85	51	16	1	1.00±0.00	25.00±0.00	984	97.97±0.45
179/2-DPP		52	32	2-8	2.50±0.37	24.75±0.19	157-537	2.61±1.27-91.81±1.18
179/2 ₁₂	Mo87	51	20	1	1.00±0.00	25.00±0.00	217-693	40.09±3.33-92.93±0.97
356/8-DPP		52	18	2	1.67±0.18	25.17±0.09	401	61.35±2.43
356/8 ₅	Mo58	51	33	1-3	1.12±0.08	24.94±0.04	685	81.02±1.50
356/8 ₆	Mo59	51	24	1	1.00±0.00	25.00±0.00	222-1292	2.25±1.00-70.12±1.27
356/8 ₇	Mo60	51	22	1	1.00±0.00	25.00±0.00	307	38.44±2.78
356/8 ₈ -unknown karyotype		-	-	-	-	-	452	53.98±2.34
356/8 ₈ -14	Mo79	51	22	1	1.00±0.00	25.00±0.00	666	92.94±0.99
356/8 ₈ -15	Mo80	51	20	1	1.00±0.00	25.00±0.00	447	99.78±0.22
356/8 ₈ -5	Mo84	51	39	1-3	1.41±0.13	24.79±0.07	322	98.14±0.75
356/8 ₈ -2	Mo91	51	19	1-3	1.11±0.11	24.95±0.05	65-726	53.85±6.18-89.81±1.12
1067/9 ₃ -2 ₆ -DPP		52	10	2-14	7.80±1.38	22.10±0.69	-	-
1067/9 ₃ -2 ₂₂	Mo89	51	44	1-3	1.09±0.06	24.95±0.03	1145	97.64±0.45

Table 4. Chromosome pairing at metaphase I observed in PMCs and pollen fertility in the cotton desynaptic parental (DPPs) and their monosomics (Mo) progenies. Bold faced rows are parental desynaptic plants.

One unique desynaptic plant-356/8 (row number/plant number in M_1 generation in field) was observed from the desynaptic progenies studies. This plant produced monosomics in high frequency with a small size of univalents and strong phenotypic differences, suggesting monosomy for different chromosomes of cotton genome. In previous we identified two new monosomics (Mo30 and Mo67) using progeny of translocation plants [33].

Meiotic metaphase I analysis of 94 cotton primary monosomics showed modal chromosome pairing with 25 bivalents and univalent in 38 plants. Fifty monosomic plants were characterized with the presence of additional univalents side by side bivalents. Thus, in 34 monosomics, the formation of three univalents in some PMCs was observed due to lack of pairing of single pair of chromosomes. Three monosomics formed five univalents in some PMCs suggesting the absence of pairing in two chromosome pairs. Another five monosomics were characterized with the presence of unpaired chromosomes in 20 – 30% PMCs. In 8 chromosome deficient plants, a strong desynaptic effect was detected as they formed from 3 to 11 univalents in 40 – 60% PMCs studied. None of the studied PMCs in the monosomic plant Mo52 revealed normal chromosome pairing because it characterized with the presence of 3 to 15 univalents. The variation in the number of univalents could be explained by different expression of synaptic genes in different cells [44]. Later, disomic desynaptic plants were found in the progeny of two plants, which produced up to 52 univalents in PMCs. This finding confirmed our hypothesis of independent parallel mutations in desynaptic genes of original plants.

Some of monosomic plants showed (Mo6, Mo7, Mo19, Mo30, Mo56, Mo61, and Mo62) univalents, bivalents and trivalents (from 0.04 ± 0.04 to 0.12 ± 0.06 in average per cell) formed at metaphase-I of meiosis. Those results suggested association of univalent with two homeologous chromosomes. Such trivalents formed by pairing of homologous chromosomes were also found in other monosomic plants [50]. Moreover, two of them (Mo56 and Mo61) were also characterized with additional univalents. The other 12 cotton primary monosomics showed quadrivalent associations with different frequencies suggesting heterozygosity for their translocation. Analysis of the sizes of monosomes revealed medium univalent size in 44 monosomics (Fig. 13 C, D); whereas there were 22 monosomics with large univalents (Fig. 13 A, B). The number of monosomics having small univalents was slightly higher (27); moreover, among these, 6 monosomics with very small univalents were detected (Fig. 13 E, F). Therefore, according to a preliminary assignment of monosomes considered on the basis of their sizes to the subgenomes, 22 large monosomes can be assigned to the A_t -genome and 27 monosomes of small sizes to the D_t -genome.

It is known that only three chromosome pairs of *G. hirsutum* have long arms that are two or three times the length of the short arms [51]. Monosomes of medium sizes demand special translocation tests with subgenome and chromosome number assignment of the translocated chromosomes. The analysis of subgenome assignment of unidentified monosomes of medium sizes showed the A_t subgenome location [52] and significant deviation from the expected 1:1 ratio of the A_t -subgenome monosome number to the D_t -subgenome ones. This observation implied that preferential loss of the A_t -subgenome chromosome was caused by specific genetic regulation system of chromosome disjunction and was not due to size of monosomes [52]. In

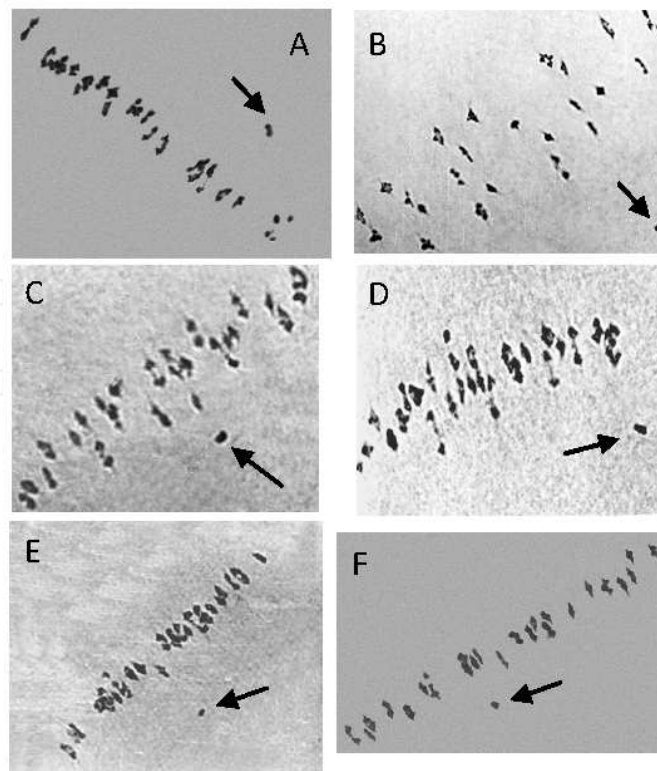


Figure 13. Meiotic configurations in monosomic lines in cotton *G. hirsutum*. Meiotic metaphase I cells showing 25 bivalents and 1 univalent in (A) Mo13 and (B) Mo16 (large univalent size); (C) Mo11 and (d) Mo4 (medium univalent size); (E) Mo80 and (F) Mo89 (small univalent size). The arrows point to the univalents. Note that the background of figures was cleaned using Adobe Photoshop CSS extended version 12.

our experiments, we detected a nearly 2 : 1 ratio of the A_t -to the D_t -subgenome monosomes for all that monosomes of medium sizes to be from A_t -genome. The ratio observed us is not significantly different from the ratio given by Myles and Endrizzi [52]. This confirms a greater tolerance of *G. hirsutum* to loss of the large A_t -genome chromosome than the small D_t -genome chromosomes.

Analysis of the tetrads was carried out for 87 primary monosomics of our collection. Most of the monosomics (73 or 83.91%) had high meiotic index (more than 90%) than that of the control plants ($95.11 \pm 0.46\%$). That indicates regular univalent chromosome disjunction. Fourteen of the monosomics (16.09%) were characterized with lowering of meiotic index from 89.33% (Mo74) to 68.32% (Mo4). Moreover, 10 of the monosomics had a smaller reduction of meiotic index (to 80%) compared others two monosomics (Mo4 and Mo16). These monosomics were induced in M_1 generation by pollen gamma-irradiation in doses of 20 and 25 Gy, leading to strong meiotic index reduction (to $68.32 \pm 1.10\%$ and $76.07 \pm 0.93\%$, respectively). We also observe an increase of percentage of tetrads with micronuclei (to $6.87 \pm 0.60\%$ and $21.56 \pm 0.90\%$ respectively) in comparison with the control line ($1.42 \pm 0.25\%$). Two other monosomics (Mo88 and Mo90), selected from M_3 generation treated by thermal neutrons and pollen with gamma-rays were characterized with different meiotic

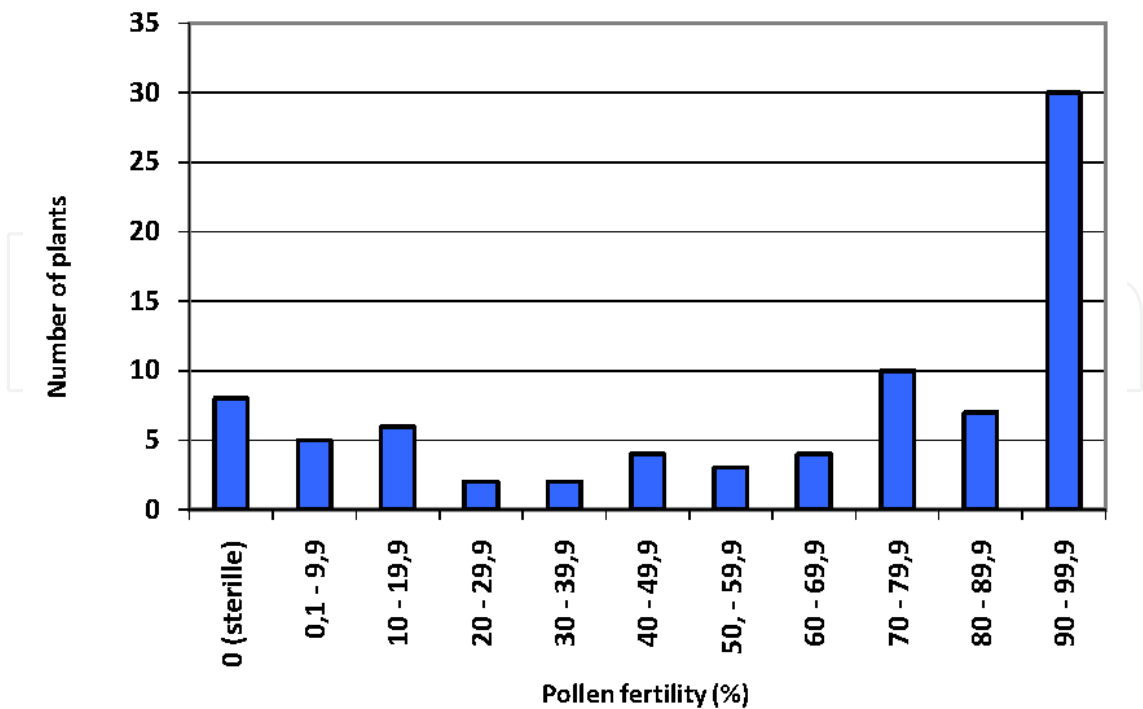
index in various buds. Variation limits were also observed for the number of tetrads with micronuclei.

Meiotic index decrease in 6 monosomics (Mo16, Mo28, Mo52, Mo74, Mo88 and Mo90) could be explained because of the presence of a additional univalents at meiotic metaphase I. In contrast, meiotic index decrease in 4 monosomics (Mo8, Mo21, Mo23 and Mo57) was connected with simultaneous translocation heterozygosity that led to chromosome disjunction disturbances and the production of tetrads with micronuclei. However, meiotic index decrease in 3 monosomics with the modal chromosome pairing (Mo4, Mo34 and Mo37) and increase of number of tetrads with micronuclei In Mo4 (to $6.87 \pm 0.60\%$) directly demonstrated disturbances in monosome disjunction and imbalanced gamete formation. Therefore, the low frequency of tetrads with micronuclei in cotton monosomic argues for stability of monosomes, which seldom undergo irregular division (misdivision) of univalent centromeres [43]. This is confirmed by the fact that we found only 9 monotelodisomics and one isochromosome in more than 1000 cytologically examined plants of the progeny of various monosomics from our cytogenetical collection. These results are in contrast with earlier data on the degree of chromosome lagging in wheat monosomics, where the frequency of tetrads with micronuclei varied from 34.1 to 65.2% [53].

Pollen fertility after acetocarmine staining was studied in 93 primary cotton monosomics, isolated mainly from different types of irradiation. High pollen fertility was detected only in 30 plants with chromosome deficiencies that pointed out probable early haplo-deficient microspore abortion prior to mature pollen stage. Remaining monosomics were characterized with pollen fertility decrease. Thus, 17 monosomics had small lowering of pollen fertility (to 70%), 11 – semisterile pollen (to 40%) and 15 – strong pollen fertility reduction (to 5%) (Fig. 14). Pollen sterility was established in 6 monosomics (Mo5, Mo7, Mo10, Mo44, Mo45 and Mo47) derived from M_1 generation after irradiation of pollen and in two monosomics (Mo57 and Mo74) isolated after thermal neutron seed irradiation. Monosomics Mo5 and Mo44 did not produce any seeds from self-pollination and intercrossing that suggest their complete sterility. In 11 monosomics pollen fertility was varied among different flowers on the same plant; moreover, the variation limits were strongly differed. Reproduced monosomics from the 3 other families (Mo22, Mo39 and Mo46) also had pollen fertility variation in different flowers on the same plant from semisterile or low to reduced pollen fertility.

The reproduction of the monosomic plants was studied in the self-pollination and outcrossed progenies under the field and greenhouse conditions. Comparative analysis of the cotton monosomics produced both in the field and greenhouse revealed distinct morphological differences in comparison with disomic sibs. As a result, monosomics were reproduced in 18 generations under field condition. However, we did not analyze most of the progenies and determine exact transmission frequency in the field due to limited space, time and cost. Thirteen of 18 reproduced later under greenhouse condition whereas five monosomics (Mo22, Mo36, Mo39, Mo46 and Mo53) plants did not produce daughter monosomics.

The progenies of 81 different monosomics were studied in the greenhouse. All monosomic families strongly differed in number of plants studied, and in only 18 families were all



Note: the remaining 11 monosomics were not included in the histogram due to their varied pollen fertility level in different flowers.

Figure 14. Percentage distribution of pollen fertility for 82 cotton monosomic plants.

progenies cytologically examined for monosome transmission rate (Table 5). This demonstrated a large variation in transmission rate from high (44.44% in Mo16 and Mo84) to very low (1.79% in Mo34). The highest transmission rate (from 30.43% in Mo72 to 44.44% in Mo16 and Mo84) was observed in 12 monosomic plants (Mo16, Mo31, Mo58, Mo59, Mo62, Mo66, Mo71, Mo72, Mo77, Mo82, Mo84 and Mo90). Results suggested frequent transmission of haplo-deficient gametes. On the other hand, 12 other monosomics (Mo3, Mo4, Mo9, Mo15, Mo34, Mo35, Mo40, Mo41, Mo56, Mo61, Mo67 and Mo85) had the lowest transmission rate (from 1.79% in Mo34 to 9.38% in Mo67) due to rare $n-1$ gamete transmission that demanded to use a large population for their recovery. The remaining 26 monosomic plants were transmitted with medium range of frequency (from 14.29% in Mo10 and Mo74 to 29.41% in Mo11). Significant variability in transmission rates could be explained by differences in the viability of haplo-deficient gametes involving specific chromosomes. Theoretically, after selfing monosomics must produce progenies with $2n$, $2n-1$ and $2n-2$ chromosome number in the ratio 1:2:1, but, in fact, cotton nullisomic gametes with $2n-2$ are nonviable whereas n and $n-1$ gametes form in unequal frequencies because of lower haplo-deficient gamete viability and their incompetitiveness in comparison with normal gametes. Thus, all the differences in detected transmission rates involve deficiencies in various chromosomes of the cotton genome. Nevertheless, transmission rate similarities in some monosomes in our collection could indicate identities that should be explored.

Mo	Total no. of plants	No. of studied plants	Discomics (26II)	Monotelodisomics (25II+1t)	Monosomics (25II+1I)	Trans-mission (%)	No. of progenies
Transmission of monosomes studied in outcrossed progenies							
(Mo1)*	6	6	5	0	1	16.67	1
(Mo2)	6	6	5	1	1	16.67	1
Mo3	29	24	23	0	1	4.17	3
Mo4	45	29	27	0	2	6.9	2
(Mo10)	7	7	6	0	1	14.29	2
Mo11	52	34	24	0	10	29.41	4
Mo13	27	21	18	0	3	14.29	3
Mo15	44	42	41	0	1	2.38	5
(Mo28)	5	5	4	0	1	20.00	1
Mo34	64	56	54	1	1	1,79	3
Mo41	15	14	13	0	1	7,14	2
Mo56	26	26	24	0	2	7.69	4
Mo63	19	13	11	0	2	15.38	2
(Mo74)	12	7	6	0	1	14.29	1
Transmission of monosomes studied in selfed progenies							
Mo7	29	19	14	0	5	26.32	2
Mo9	48	34	32	0	2	5.88	3
Mo16	22	18	10	0	8	44.44	3
Mo17	33	31	24	0	6	19.35	9
Mo19	38	31	24	1	6	19.35	4
(Mo27)	9	9	7	0	2	22.22	2
Mo31	25	25	16	0	9	36.00	5
Mo35	24	23	21	0	2	8.70	3
Mo38	17	17	14	0	3	17.65	2
Mo40	33	33	32	0	1	3.03	2
Mo42	30	25	21	0	4	16.00	2
(Mo48)	11	11	9	0	2	18.19	3
Mo50	37	26	20	0	6	23.07	3
(Mo58)	5	5	3	0	2	40.00	1
Mo59	22	22	13	0	9	40,91	1

Mo	Total no. of plants	No. of studied plants	Discomics (26II)	Monotelodisomics (25II+1t)	Monosomics (25II+1I)	Trans-mission (%)	No. of progenies
Mo60	16	14	10	0	4	28.57	3
Mo61	41	18	16	1	1	5.56	3
Mo62	61	24	17	0	11	35.48	3
Mo66	31	31	20	0	11	35.48	3
Mo67	40	35	32	0	3	9.38	4
Mo69	18	18	13	0	5	27.78	2
Mo70	18	18	15	0	3	16.67	3
Mo71	20	20	13	0	7	35.00	2
Mo72	23	23	16	0	7	30.43	2
Mo73	28	24	18	0	6	25.00	2
Mo75	35	15	11	0	4	26.67	3
Mo76	31	18	14	0	4	22.22	4
(Mo77)	22	10	6	0	4	40.00	1
Mo79	31	22	16	0	6	27.27	3
Mo80	48	20	17	0	3	15.00	3
Mo81	21	12	9	0	3	25.00	2
(Mo82)	17	11	7	0	4	36.36	2
Mo84	31	18	10	0	8	44.44	2
Mo85	48	26	25	0	1	3.85	2
Mo89	47	21	17	0	4	19.05	3
Mo90	16	16	11	0	5	31.25	1

*Families shown in parenthesis are too small to provide a very informative assessment.

Table 5. Transmission of the monosomes in the progenies of cotton monosomics (Mo) under greenhouse condition

In cytogenetic analysis, 30 out of 52 cotton monosomic lines showed modal chromosome pairing with 25 bivalents plus one univalent at metaphase-1 of meiosis. The remaining 20 monosomic lines were characterized by the presence of additional univalents in PMCs; moreover, three lines (Mo10, Mo11 and Mo39) had highest frequencies of such univalents (from 1.21 ± 0.10 to 1.33 ± 0.08 in average per cell, respectively). The line Mo4 was characterized by the presence of rare trivalents (0.12 ± 0.06 in average per cell) that suggested pairing of the monosomic chromosome with homoeologous chromosome. Appearance of additional univalents in the monosomic lines was seen previously in cotton. Homozygotization of

daughter monosomic genotype led to meiosis stabilization and absence of additional univalents in subsequent generations.

Monosomic lines were also distinguished by sizes of the univalents. Thus, 8 lines were characterized with univalents of large sizes, 29 monosomic lines had univalents of medium sizes, 11-had small univalents. The remaining 4 monosomic lines had extremely small univalents that suggested a different sub-genome origin and genetic non-uniformity. In three monosomic lines (Mo1, Mo9 and Mo46), the sizes of univalents differed various in the parental and daughter monosomics, underlining the possibility of univalent shifts in progeny.

Analysis of tetrads of microspores showed a high meiotic index in the majority of the monosomic lines with the exception of the line Mo84 which varied in both meiotic index (from $49.90 \pm 1.12\%$ to $95.48 \pm 0.27\%$) and tetrads with micronuclei (from 12.44 ± 0.74 to $0.53 \pm 0.10\%$) in different buds. The meiotic index variation led to variation in pollen fertility (from $65.14 \pm 1.45\%$ to $94.46 \pm 0.36\%$) within individual flowers of the same plant. It should be noted that lower meiotic index was recorded in wheat monosomic lines and a high percentage of tetrads with micronuclei confirmed that univalents frequently lagged during chromosome disjunction [54].

Pollen fertility analysis of cotton monosomic lines after acetocarmine staining showed high pollen fertility in the majority of the lines. Only line Mo10 was characterized with strong lowering of the character (to $19.35 \pm 2.37\%$) that suggested its partial sterility of chromosome deficient pollen. Six other lines (Mo22, Mo34, Mo39, Mo46, Mo84 and Mo89) showed variation in pollen fertility in different flowers within the same monosomic plants. In the three parental monosomics (Mo22, Mo39 and Mo46) variation in pollen fertility among different flowers within the same plants was also observed (13.43 - 91.37% ; 2.53 - 34.21% ; 2.10 - 92.46% , respectively). The ranges of variation in pollen fertility were wider in two monosomics (Mo22 and Mo46). A similar effect detected in daughter monosomics, confirmed the genetic determination of such variation and suggested possible chromosome localization of the gene(s) for male gametophyte viability in the deficient chromosomes. It is known that the majority of cotton chromosome deficiencies are not transmissible via pollen due to non-functionality of chromatin-deficient pollen [54]. Besides, Kakani et al. [19] reported that gene(s) responsible for pollen spine development were located on long arm of chromosome 12 using the advanced technique of confocal laser scanning microscopy and substitution lines.

A study of the morphology of cotton monosomic plants revealed the specific influence of monosomy on many characters that were differentiated them from disomic sibs. Such characters were thin stem, feeble leafing, small leaves, short internodes, crooked sympodia, small flowers and bolls, as well as deformed and obligospermous bolls. At the same time, 4 monosomic lines (Mo35, Mo36, Mo40 and Mo50) looked like disomic sibs. Although the majority of the monosomic lines had a compact bush, 10 lines (Mo3, Mo7, Mo11, Mo31, Mo35, Mo39, Mo60, Mo69, Mo73 and Mo89) were characterized by a scattered bush. Two lines (Mo7 and Mo56) differed by having a crooked sympodia and 3 other lines (Mo75, Mo76 and Mo82) had elongated internodes. In 3 lines (Mo13, Mo34 and Mo66), a dense stem pubescence was observed whereas leaf pubescence was feeble (Figure 15). Three monosomic lines (Mo16, Mo31 and Mo48) had difference in leaf sizes within the same plant

and two other lines (Mo9 and Mo76) had leaf folding in the area of the main rib or lobe division, respectively.

Four monosomic lines (Mo4, Mo10, Mo46 and Mo67) differed by having feeble budding and flowering (to 10-15 flowers during the summer) whereas three other lines (Mo22, Mo39 and Mo56) had strong budding and flowering (to 40-60 flowers during the summer) but low seed and boll set (from $10,10 \pm 0,78$ to $20,71 \pm 0,52$ per one boll). Many monosomic lines were characterized by small flowers and bracts; however, 6 lines (Mo4, Mo10, Mo16, Mo34, Mo46 and Mo48) were distinguished by a strong reduction in flower sizes (from 38mm to 48mm). Taken together, 7 monosomic lines (Mo9, Mo31, Mo39, Mo71, Mo72, Mo73 and Mo76) had large bracts (to 65x67mm for Mo9) and 5 monosomics (Mo4, Mo10, Mo34, Mo46 and Mo80) had small bracts (to 25x21mm for Mo10). Some chromosome deficient lines (Mo31, Mo72 and Mo76) differed by having a large number of bract teeth (from 14 to 18) whereas other lines had small number of bract teeth (Mo4, Mo10, Mo19, Mo34, Mo46 and Mo80) (from 8 to 12) (Figure 16). In the Mo39 line additional bracts were present, in the Mo17 the bracts were asymmetrical and in the Mo27 the bracts were deformed with feebly expressed teeth.



Figure 15. Some examples of morphology of cotton monosomic lines: (A) Mo11; (B) Mo31; (C) Mo50; (D) Mo76; (E) Mo81; (F) Mo82; (G) Mo89; (H) Mo90.

The most variability was observed for the character “presence/absence of nectarines” where in 15 monosomic lines not all bracts had nectarines, and Mo66 lacked any external nectarines. Nectarines of different sizes within a single flower were presented in 6 monosomic lines (Mo9, Mo27, Mo31, Mo39, Mo84 and Mo89) (Figure 17).

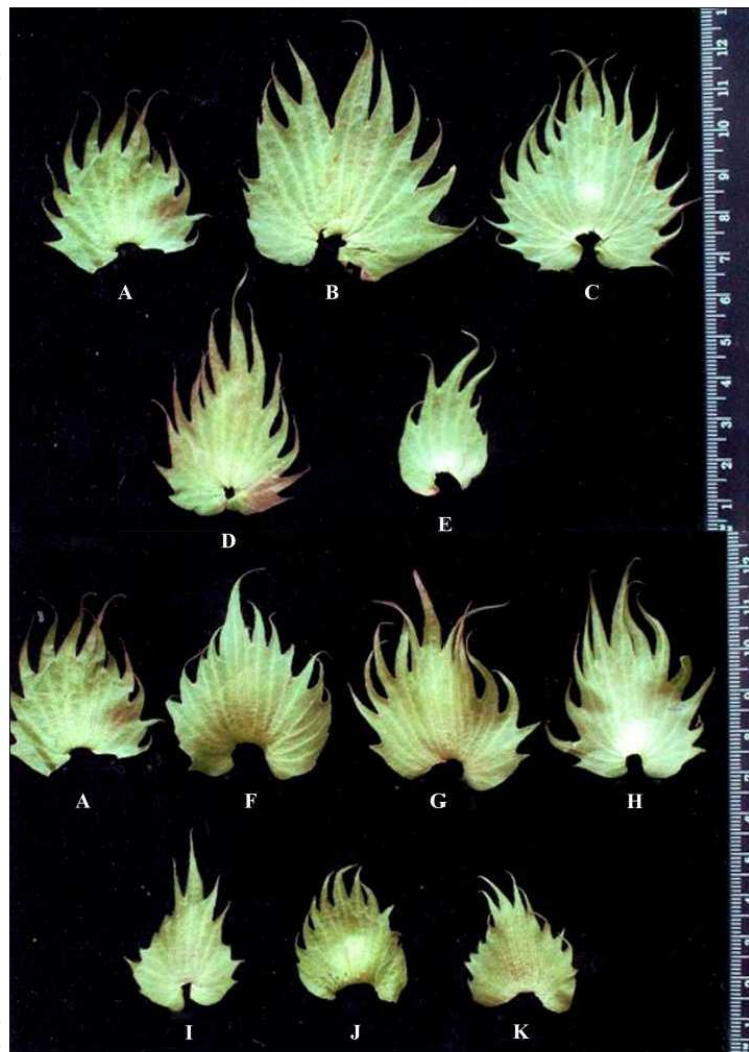


Figure 16. The bracts in the different cotton monosomic lines : (A) parental line L-458; (B) Mo39; (C) Mo72; (D) Mo31; (E) Mo66; (F) Mo84; (G) Mo89; (H) Mo81; (I) Mo88; (J) Mo4; (K) Mo92.

Monosomy had an influence on the stigma structure and sizes in a flower. Thus, there were shorter stigmata in 3 lines (Mo17, Mo19 and Mo28) and a broad “reverting” stigma in Mo39. A new phenotypic marker for cotton monosomy – “reduced” stigma was detected in Mo62. Analysis of Mo62 progeny revealed the presence of reduced stigma only in monosomic cytotypes whereas disomic ones had normal stigma as did the control (Figure 18). This trait it makes possible to distinguish cytotypes within the progeny without cytological analysis. However, stigma reduction rate was varied in different flowers within the same plant: a little reduction stigma (to 7-9 mm); medium reduction (stigma to 2-6 mm), and strong reduction

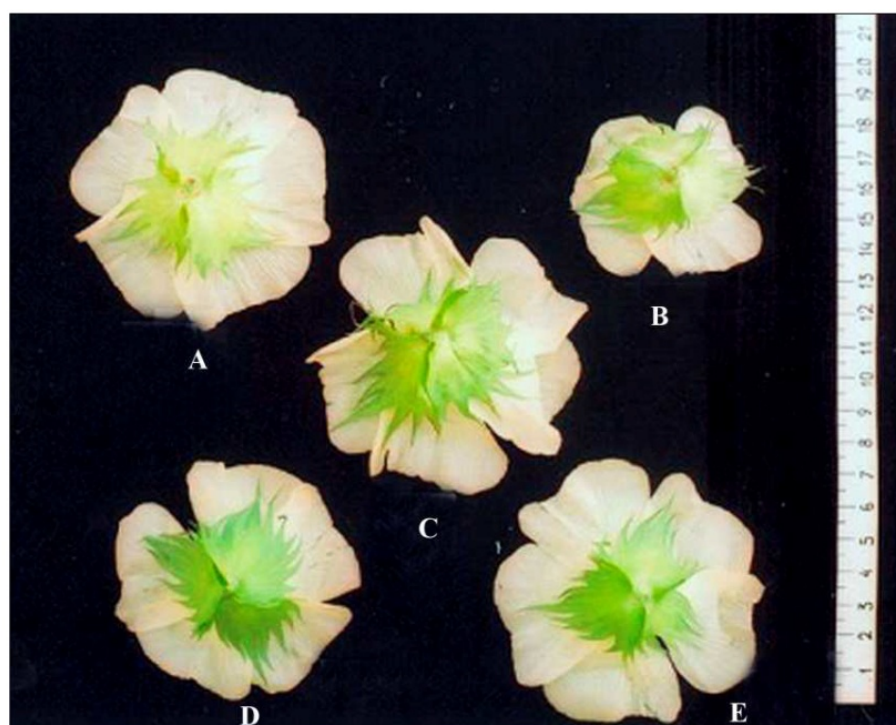


Figure 17. Outside view of nectaries in some cotton monosomics: (A) parental line L-458; (B) Mo13; (C) Mo39; (D) Mo71; (E) Mo72.

(stigma to 1 mm). Moreover, as a rule, strongly reduced stigmas were located inside the staminate column. Besides flowers with reduced stigma, there were flowers in which the stigma was closed inside the stylar tissue. A dependence of stigma reduction rates related to the seasons of a year was also established.

All daughter monosomics of Mo62 were fertile both as male and female but had lower seed number per a boll (22.30 ± 1.83) and lower seed set (76.90 ± 2.47 %) in comparison with the parental line L-458 (34.40 ± 0.62 and 89.81 ± 1.55 , respectively). A monosome of *G. hirsutum* with a strong reduction of stigma but still fertile, has not been described. Thus the monosome in Mo62 for the chromosome of cotton genome could be new.

The most important changes due to monosomy concerned sizes and shapes of bolls as majority of them formed smaller round bolls almost ranging from spherical to elongated bolls with beaks or without beaks compared to control plants. Many of the bolls of monosomics were ribbed or deformed due to a number of abortive ovules and immature seeds (Figure 19). As a result, the number of seeds per boll and seed set were lower in all monosomic lines (from 9.50 ± 1.62 in Mo13 to 32.61 ± 3.99 % in Mo76) in comparison with the parental line (34.40 ± 0.62 and 89.81 ± 1.55 , respectively). Mo4 was characterized with variation of boll sizes within the same monosomic plant and also the fruit occurred in clusters. Flowers and fruit clusters were also observed in Mo19. Mo66 was distinguished by a large broad beak at the top of an ovoid boll (Figure 19 D). Thus, it was shown that an individual chromosome deficiency had a specific influence in plant morphology and that some of them had unique marker characters. However, the clear similarity both morphological and cytogenetic features in some monosomics of our

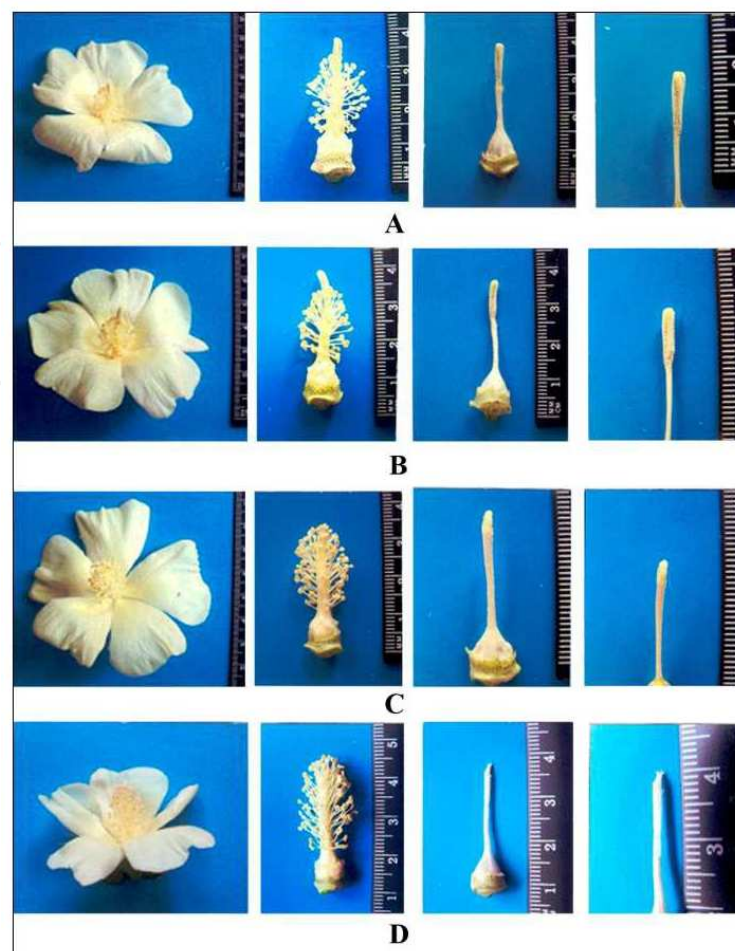


Figure 18. The flowers of the different cytotypes from progeny the monosomic line Mo62: (A) parental line-L-458; (B) disomic cytotype with normal stigma; (C) monosomic cytotype with medium reduction of the stigma; (D) monosomic cytotype with strong reduction of the stigma.

collection suggested probable redundancy for the same monosomic chromosomes among the plants.

Many small chromosomes presence in karyotype analysis of tetraploid cotton *G. hirsutum* and absence of distinctive morphological markers for the chromosomes make it impossible to distinguish and identify chromosomes in karyologic analysis. Therefore, we identified monosomes to be specific chromosomes of the cotton genome using the known translocation test on hybrids of monosomics with translocation lines from the Uzbek Cytogenetic Collection (Table 6). Analysis of hybrid chromosome pairing was used to reveal monosomic translocation F_1 hybrids and to study “critical configurations”. The recently developed 28 translocation lines (Tr1-Tr28) from our collection were used for monosome identification according to the method described previously [6].

Eleven monosomics (Mo3, Mo10, Mo11, Mo19, Mo27, Mo39, Mo48, Mo53, Mo56, Mo73 and Mo85) were associated with the chromosomes of seven translocation lines (Tr1, Tr3, Tr5, Tr8, Tr11, Tr12 and Tr16) as chromosome pairing of 24 bivalents plus one trivalent was observed



Figure 19. The bolls of the different cotton monosomic lines: (A) parental line L-458; (B to H) – Mo72; Mo31; Mo66; Mo60; Mo50; Mo39; Mo16 and (I to R) Mo80; Mo4; Mo92; Mo89; Mo81; Mo76; Mo62; Mo75; Mo87; Mo88.

in PMCs of the F_1 monosomic hybrid plants (Fig. 20). We also identified four monosome pairs (Mo10 and Mo73; Mo39 and Mo56; Mo48 and Mo53; Mo11 and Mo19) that were associated with the translocation lines Tr3, Tr5, Tr12 and Tr16, respectively (Table5). Thus, three of the above-mentioned monosome pairs (Mo10 and Mo73, Mo39 and Mo56, Mo11 and Mo19) involved the same chromosomes with the each pair. In future analyses, hybrids from crosses of the monosomics and other translocation lines, involving the same chromosomes, will confirm our interpretation about the missing chromosome or chromosome segment and identification of the monosomic line. However, there is evidence for monosomes Mo48 and Mo53 that nonhomologous as the chromosomes from two different sub-genomes all involved with translocation line Tr12.

Monosomics	Translocation lines							Total number of crosses tested
	Tr1	Tr3	Tr5	Tr8	Tr11	Tr12	Tr16	
Mo3			-		+	-		4
Mo7			-	-		-	-	7
Mo10		+	-	-				3
Mo11	-	-	-	-	-	-	+	14
Mo13	-					-	-	13
Mo19		-	-				+	5
Mo27			-	+				5
Mo31	-	-	-	-	-	-	-	25
Mo35				-				3
Mo36								3
Mo38	-		-	-				9
Mo39			+	-				6
Mo41					-			1
Mo48						+		4
Mo50	-	-	-	-		-		19
Mo53				-		+		2
Mo56			+	-		-	-	8
Mo60			-	-			-	9
Mo62	-		-					6
Mo66	-	-	-	-	-	-	-	12
Mo67	-							2
Mo69	-		-	-	-	-	-	21
Mo70		-	-		-	-	-	11
Mo71	-	-			-	-	-	12
Mo72	-	-						9
Mo73	-	+	-	-	-	-	-	14
Mo75	-	-	-	-	-		-	18
Mo76	-		-					9
Mo77								6
Mo79	-		-	-			-	10
Mo80			-		-			4
Mo81	-		-		-	-	-	12
Mo84								1
Mo85	+					-		4
Mo89							-	5

Note: +associated, -independent

Table 6. Cytological test for indentification of the monosomes with the help of translocation lines.

We had isolated 4 monosomics (Mo70 – Mo73) from the progeny of the same desynaptic plant and proposed possible monosomy for different nonhomologous chromosomes of the cotton genome. Indirect confirmation was available with the detection of monosome Mo73 homology and one of the chromosomes involved into interchanges in the line Tr3 whereas the other three monosomes from the progeny of the same desynaptic plant (Mo70, Mo71 and Mo72) did not have any chromosomes in common in the Tr3 interchange. Another monosome (Mo85), isolated from the other desynaptic progeny, showed homology with a chromosome involved in an interchange with Tr1. This test revealed that the chromosomes of Tr1 were rarely involved in translocations. Translocation line Tr1 had common chromosomes only with two lines – Tr2 and Tr20 with multiple interchanges [41]. This verified our assumption that new or rare monosomes would occur in progenies of desynaptic forms of cotton [33].

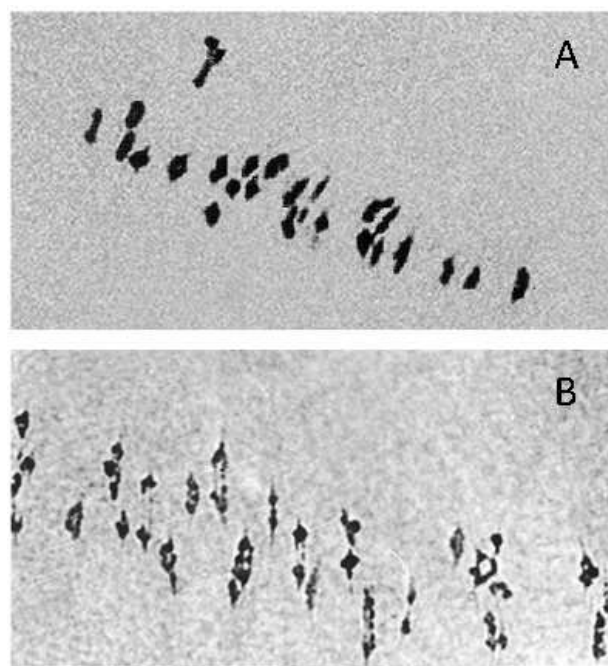


Figure 20. “Critical configurations” of the chromosomes at the meiotic metaphase I cells showing 24 bivalents and 1 trivalent in cotton F_1 plants from crosses the monosomics x translocation lines: (A) Mo19 x Tr16; (B) Mo85 x Tr1. The arrows point to the univalents. Note that the background of figures was cleaned using Adobe Photoshop CSS extended version 12.

Translocation tests involving other 24 monosomic lines have not yet revealed any homology of the monosomes and the chromosomes involved in interchanges because they showed detections of chromosome pairing with 23 bivalents plus one univalent plus one quadrivalent (Figure 21). However they did demonstrate the differences in the studying level of the lines as well as depended on transmission rates of the monosomics in hybrid progenies. There is an evidence of the comparative rareness of other monosomes from our collection.

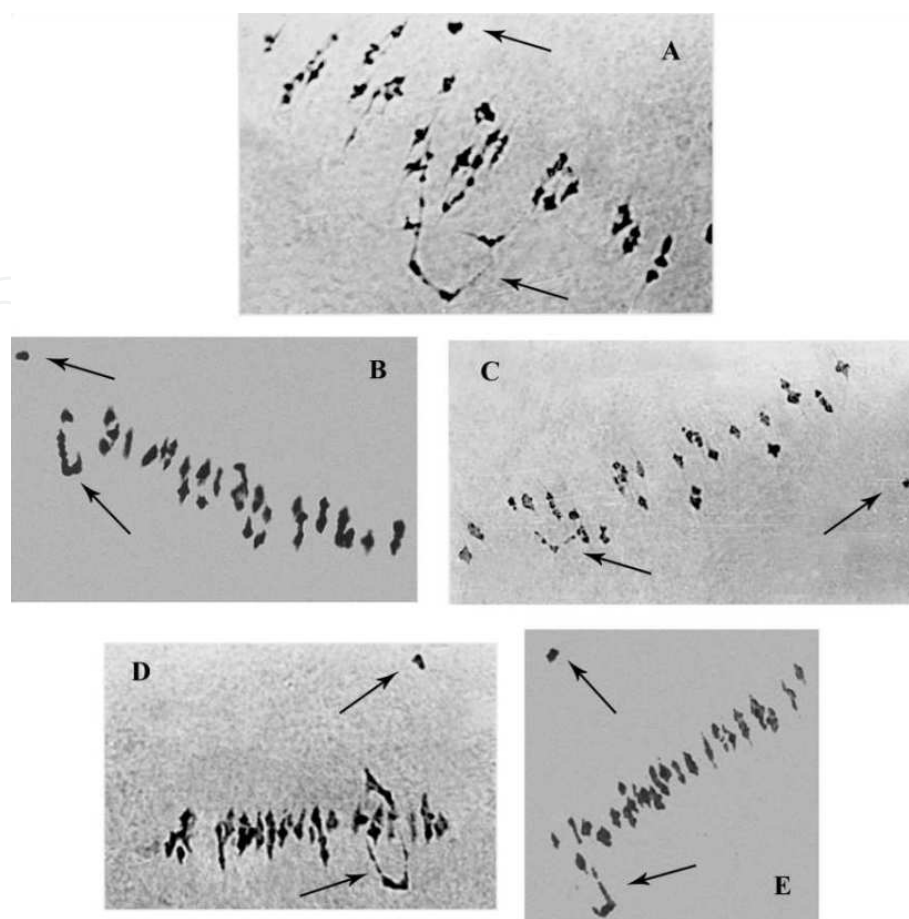


Figure 21. “Critical configurations” of the chromosomes at the meiotic metaphase I cells showing 23 bivalents and 1 univalent and one hexavalent (A) and 23 bivalents and 1 univalent and one quadrivalent (B-E) in cotton F_1 plants from crosses the monosomics x translocation lines: (A) Mo13 xTr2; (B) Mo56 x Tr6, (C) Mo75 xTr16; (D) Mo77 x Tr21; (E) Mo77 x Tr25. The arrows point to the univalents, quadrivalents and hexavalent. Note that the background of figures was cleaned using Adobe Photoshop CSS extended version 12.

6. Monotelodisomics, monoisodisomics and haploids in cotton *G. hirsutum* L.

Other type of chromatin deficiency namely monotelodisimics are characterized with absence of a chromosome arms. As a result 25 normal bivalents and one heteromorphic bivalent with arm deficiency formed in meiosis. There are 11 monotelodisomics following pollen and seed irradiation and 9 from different monosomic progenies in our collection at present time. Misdivision of a chromosome via centromere region followed by irradiation produced telocentric chromosome formation. Centromere inactivation caused loss of a chromosome arm. Telosome pair was detected as two different size univalents with various frequencies in the monotelodisomics (to 1.85 ± 0.15 in average per cell). A high frequency of heteromorphic bivalents (to 0.95 ± 0.05 in average per cell) was registered in monotelodisomics (Figure 22). Among the plants with arm deficiencies three had also a translocation.

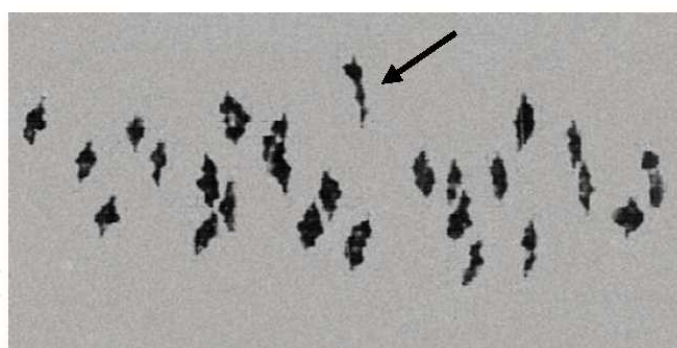


Figure 22. Meiotic configurations in monotelodisomic plant in cotton *G. hirsutum*. Meiotic metaphase I cells showing 25 normal bivalents and 1 heteromorphic bivalent with arm deficiency in plant M₁-1608/2. The arrow point out to the heteromorphic bivalent. Note that the background of figures was cleaned using Adobe Photoshop CSS extended version 12.

Monotelodisomics with the translocation had a telocentric chromosome as univalent in the majority of PMCs. As it is known, telocentrics for short arms are less often paired with normal homologous chromosomes [55]. Meiotic index was high (from 81.86 ± 0.85 to 99.56 ± 0.16), but the pollen fertility reduced in the monotelodisomics.

Isochromosome formation was also connected with damaging action of radiation to centromere chromosome regions. As a result of centromere inactivation instable telocentric formed its arm developed on 180° , gave an isochromosome. Three monoisodisomics our collection were differed with isochromosome pairing at metaphase I of meiosis. If one of them had heteromorphic in most PMS (to 0.96 ± 0.04) in other plants the isochromosome was often as univalent (to 0.91 ± 0.22 an average on PMS). In spite of high Mi (to 98.55 ± 0.25) in two monoisodisomics pollen fertility was reduced.

Deficiencies for one chromosome arm occurred in the progenies of 9 monosomics. Thus, in four monosomic progenies (Mo2, Mo19, Mo34 and Mo61) that differed with respect to monosome transmission rates, monotelodisomics were produced due to univalent instability and resulted in misdivision. In the progenies of Mo6, Mo21, Mo22, Mo49, Mo54 and Mo68 daughter monosomics failed to produce, but monotelodisimics (from the progenies of Mo6, Mo21, Mo22, Mo49 and Mo68) and a monoisodisomic plant (from the progeny of Mo54) were detected. The results suggested an irregular univalent chromosome centromere misdivision in the parental monosomics that led to a single chromosome arm missing and formed either telocentric or isochromosome in the case of an arm doubling. Our results demonstrated the rather rare occurrence of telo- and isochromosomes in the monosomic progenies studied, which showed univalent misdivision to be rare.

Haploid plants of cotton are characterized by presence of 26 univalent chromosomes and by significant decrease in vigour and fertility, size of lives, bolls and flowers. Our collection includes four haploid plants. One haploid was obtained by irradiation of the seeds by fast neutrons (1005/22), other two (1579/6 and 171/4₁₇) – by pollen irradiation in M₁ and M₂ generations and one – from monosomic progeny (175/4₁₈₋₅₇). Meiosis was studied in the microsporocytes of the haploid plants 1005/22. Among 44 studied PMCs at metaphase I only

five cells had open bivalents (to 0.14 ± 0.06 on average per cells) (Figure 23 A). Others PMCs were characterized by presence of 26 univalents (Figure 23 B, C). As results, the range of polyads observed in PMCs.

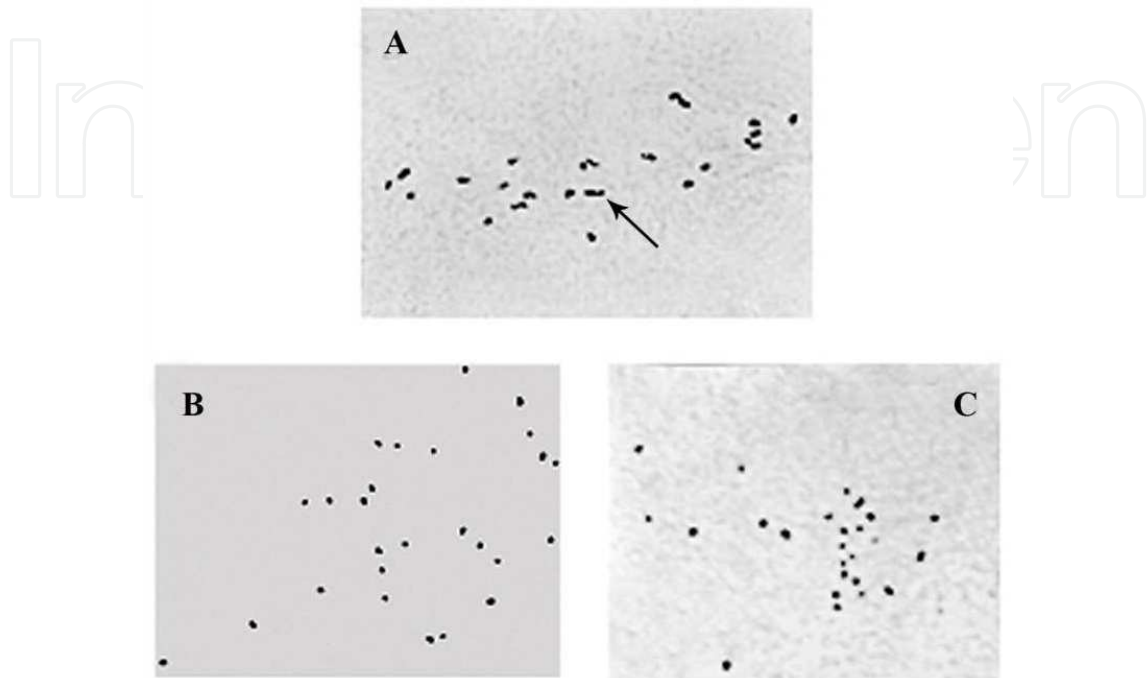


Figure 23. Meiotic configurations in haploid plant 171/4₁₇ in cotton *G. hirsutum*. Meiotic metaphase I cells showing (A) 25 univalent and 1 bivalent; (B-C) 26 univalent. The arrow point out to the bivalent. Note that the background of figures was cleaned using Adobe Photoshop CSS extended version 12.

All the 26 chromosomes remained as univalents in other two haploid plants (1579/6 and 175/4₁₈₋₅₇). Most abnormal haploid plant (1579/6) had low habit and a thin stem, compact bush, and scarce foliage. It was completely sterile. Its prominent feature was the complete absence of chromosome pairing. In all PMCs, meiosis studies revealed 26 univalents, scattered throughout the cells. Analysis of sporads revealed a significant decrease in meiotic index (to $18.97 \pm 1.31\%$), an increase in the number of tetrads with micronuclei (to $12.01 \pm 1.09\%$), and formation of abundant monads, dyads, triads, and polyads. The complete absence of chromosome pairing resulted in the formation of imbalanced and abortive gametes and pollen sterility. Haploid plant 171/4₁₇ had rare open bivalents and one trivalent in several PMCs. Some PMSs of the two haploid had 26 bivalents. Non-disjunction during premeiotic mitosis could give rise to such a diploid cell.

Analysis of sporads revealed a significant decrease in meiotic index (to $13.12 \pm 0.92\%$), the decrease in the number of tetrads with micronuclei (to $3.02 \pm 0.46\%$), and formation of abundant pentads ($16.51 \pm 0.01\%$), hexads ($22.33 \pm 1.13\%$), heptads ($17.17 \pm 1.02\%$) and oktads ($10.46 \pm 0.83\%$) (Figure 24). In three years few bolls were obtained on this haploid plant (Figure 25).

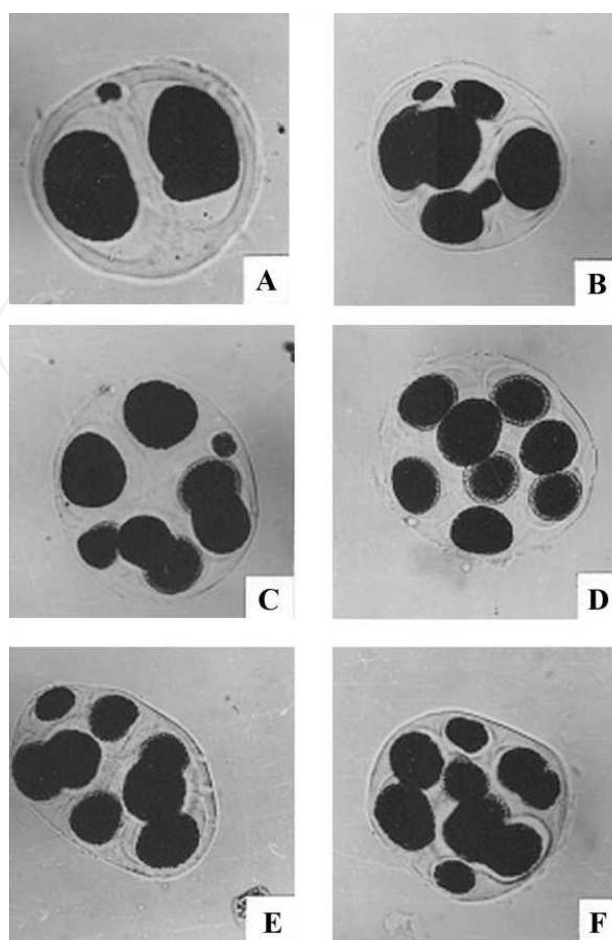


Figure 24. Meiosis in haploid plant 171/4₁₇ in cotton *G. hirsutum*. Abnormal sporads: (A) diad with micronuclei; (B) pentad with two micronuclei; (C) heksad with two micronuclei; (D and E)-oktads; (F) abnormal poliad.

Different mechanisms are proposed to explain the emergence of haploids during pollen irradiation. One of them suggests that haploids result from female parthenogenesis induced by pseudofertilization with irradiated pollen. According to another mechanism, fertilization occurs before zygotization, but the damaged paternal genome is eliminated early in development [56]. Some authors believe that haploids of *G. hirsutum* are completely sterile, whereas haploids *G. barbadense* are fertile and produce seeds after pollination with normal pollen [57]. Moreover, a line of the latter species is known that frequently produced haploids of the androgenous and matroclinous types.

7. Storage and propagation cytogenetical collection of cotton

Seeds Cytogenetical Collection of cotton maintained under room conditions (20-25⁰ C). There is no facility available for cold storage of seeds. They are placed in to parchment paper bags. Each bag has catalogue number and year of collection. Bags are stored in special metal boxes (30 x 11 cm) and boxes are placed in wooden-cases. Monosomic and translocation plants and

of their hybrids are grown at the greenhouse conditions in soil. All data collected are stored as a hard copy catalogue book that is being conversed to electronic format.

8. Location, maintenance and funding

The Cytogenetical Collection of cotton currently stored in the National University of Uzbekistan at Tashkent. It is funded by Committee for Coordination of Science and Technology Development (CCSTD) under the Cabinet Ministry of Uzbekistan.

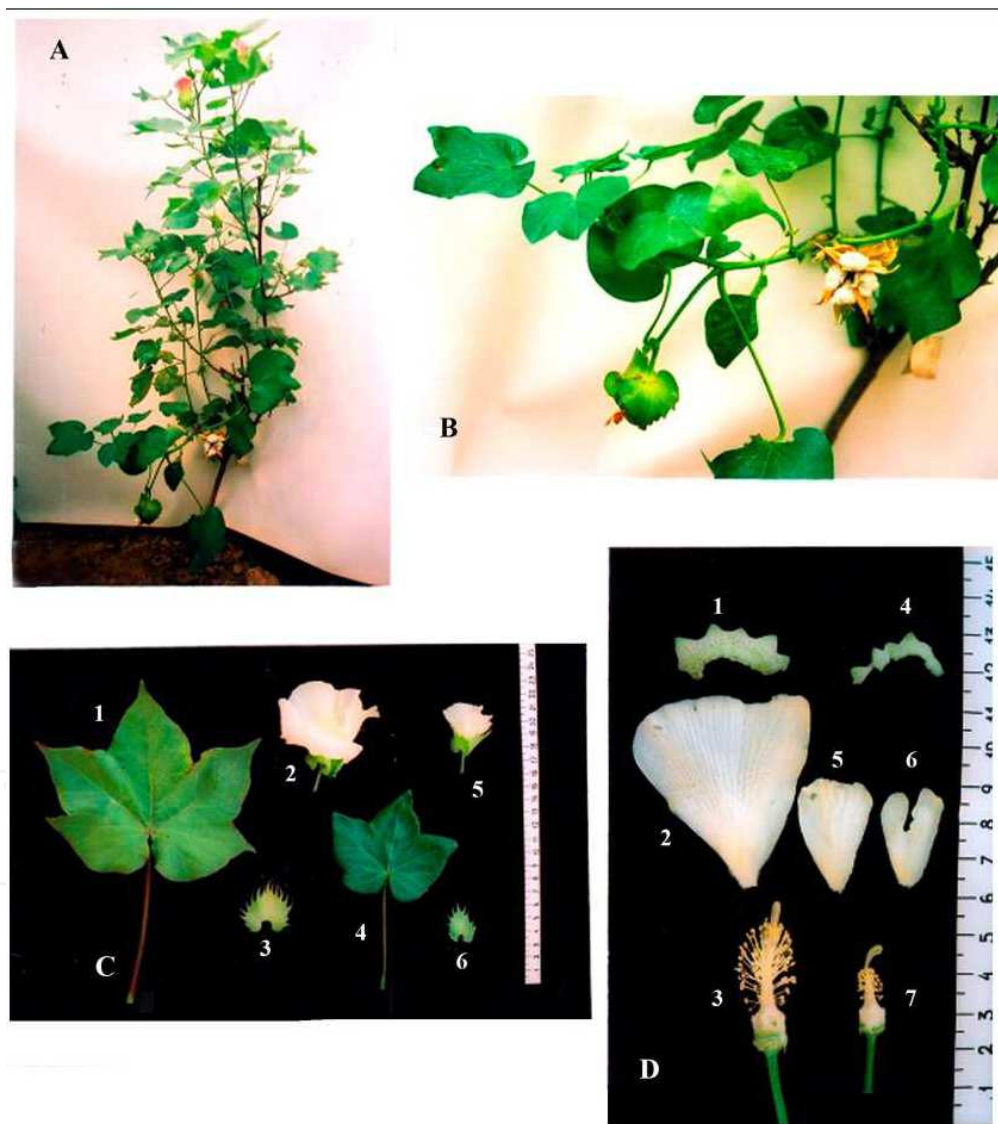


Figure 25. Haploid plant 171/4₁₇ in cotton *G. hirsutum* obtained in M₂ after pollen irradiation. (A) haploid plant; (B) fertile branch with two bolls; (C) leaf, flower and bract in parental line L-458 (1-3) and haploid plant (4-6); (D) cup, petal and staminate column in parental line L-458 (1-3) and haploid plant (4-7).

9. Conclusions

In conclusion we studied new Cotton Cytogenetic Collection adapted to the Central Asian condition in contrast Cytogenetic Collection from USA using different types of seed and pollen irradiation. We propose the presence of unique cotton aberrations involved chromosomes for absent chromosomes in American collection. The results suggested a detection of “reduced” stigma as a useful phenotypic marker for cotton monosomics which makes it possible to distinguish different cytotypes without cytological analyses. The results demonstrated of new unique desynaptic cotton plants in which progeny produced monosomics with high frequency. We observed the very occurrence of univalents misdivision probably owing to monosome stability in the unique genetic background. Our cotton monosomic lines are unique and should be a valuable cytogenetic tool not only for chromosome assignment of new marker genes and genome enrichment with new chromosome deficient plant, but also for a development of new cotton chromosome substitution lines and germplasm introgression.

Alternatively, the creation of chromosome substitution lines through crossing of each of the new monosomics with *G.barbadense* genotype (Pima 3-79) is in progress. This will serve as a foundation to apply molecular marker (e.g., SSPs) for the identification of our monosomics in hybrids with chromosome substitutions for a given monosome. At the same time, our monosomic cotton lines with initial cytogenetic characteristics, which developed using single genome background, should be useful germplasm for cotton researchers to use as material for future breeding genetic, cytogenetic and molecular-genetic investigation of cotton genome.

In future we plan to identify the chromosome deficiencies by molecular markers (SSR) to map of cotton genome. Also we will continue identification monosomic lines of our cytogenetic collection using a well-defined tester-set of translocation lines of the USA Cytogenetic Collection, kindly provided by Dr. D.M. Stelly, Texas A&M University, USA, under USDA germplasm exchange program.

Acknowledgements

This work was partially supported by research grants 38/96, 28/98, 26/2000, F.4.15 and F-5-31 from Committee for Coordination of Science and Technology Development (CCSTD) of the Republik of Uzbekistah. We thank Dr. Svetlana Polyarush and Artyom Maknyov for their help in design of the chapter.

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