BIOLOGIA PLANTARUM 45 (3): 367-373, 2002

# Cytogenetic analysis of cotton monosomics

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## Abstract

Eleven monosomics in cotton that were obtained in the progenies of three disomic desynaptic plants were cytologically characterized. The transmission of the monosomes in progeny was shown in the 26 monosomic plants. In 23 plants the frequency of monosomics was ranged between 14.29 and 41.67 %. Three monosomics usually occurred in much lower frequencies (from 3.03 to 5.00 %). Various transmission rates indirectly pointed out different monosomes as a specific chromosomes of cotton genome. Three telochromosomes and one isochromosome were isolated from the progenies of the four monosomics. Using translocation test it was recovered that seven monosomes of different monosomics are homologous to one of the chromosomes of six translocation lines of our collection.

*Additional key words*: *Gossypium hirsutum*, desynapsis, monosomes, transmission.

## Introduction

During long term studies high number monosomic plants in cotton of different origin were isolated (Brown and Endrizzi 1964, Endrizzi and Brown 1964, Galen and Endrizzi 1968, Endrizzi and Ramsay 1979, 1980). The majority of monosomes are for chromosomes 2, 4 and 6 of the A-subgenome (Endrizzi and Ramsay 1979, Edwards *et al.* 1980). Unfortunately, a complete series of 26 monosomic lines in cotton has not yet been established. From this incomplete series monosomes for 15 of the 26 chromosomes of *G. hirsutum* have been identified. These identified chromosomes are 1, 2, 3, 4, 6, 7, 9, 10 and 12 of the A-subgenome and 16, 17, 18, 20, 22 and 25 of the D-subgenome (Endrizzi *et al.* 1985). Moreover recently molecular cytogenetic techniques have been obtained and used extensively for molecular cytogenetic mapping of loci in meiotic chromosomes of cotton (Price *et al.* 1990, Crane *et al.* 1993, Reyes-Valdes *et al.* 1996, Ji *et al.* 1999).

In previous papers we gave of basic information on the origin, chromosome pairing, sizes of univalents, fertility of pollen and morphological characters of the monosomics in cotton collection of National University of Uzbekistan (Sanamyan and Musaev 1990, Sanamyan *et al.* 1996, 2000a,b). The aim of the present study was to describe of the transmission of the monosomes in progeny and of the identification of the monosomes in cotton. The more recent development of 20 translocation lines has allowed them to be used for identification of cotton monosomes (Sanamyan and Musaev 1992, 1995).

## Materials and methods

Inbred line L-458 of cotton (*Gossypium hirsutum* L.) from National University of Uzbekistan was used for creation of series of monosomic lines. Monosomics were isolated from a single cultivar population, some difference observed among them could be attributed to the difference in their monosomes. Monosomics of our collection were numbered from Mo1 to Mo84 mainly in the order that they were found. Four monosomics (Mo56, Mo60, Mo62 and Mo63) were induced by treatment of seeds by thermal neutrons in $M_1$ and $M_2$ generations. For this purpose, thermal neutron irradiation of seeds of L-458 was carried out at the biological channel reactor

*Received* 29 January 2001, *accepted* 4 July 2001.
*Acknowledgements*: This work was partially supported by research grants 26/2000 and GNM 19/2000 from the Committee for Science and Technology of the Republic of Uzbekistan.
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*VVR-SM* (Institute of Nuclear Physics of Academy of Science of Republic of Uzbekistan, Tashkent). Doses of 15, 25 and 35 Gy were used.

Monosomic plant Mo67 was isolated from progeny of a translocation plant, which also was obtained by irradiation of seeds by thermal neutrons. Seven monosomics (Mo69, Mo70, Mo71, Mo72, Mo73, Mo78 and Mo79) were found in the progenies of three desynaptic disomic plants, which were derived from irradiated materials. All others monosomics reported here were induced by treatment of pollen with γ-irradiation in $M_1$ and $M_2$ generations. For this purpose, mature pollen of L-458 was given a γ-ray treatment of 10, 15, 20 and 25 Gy ($Co^{60}$, Central Asiatic Institute of Silk, Tashkent) and thereafter was used for pollinating of the flowers that were emasculated and enclosed in parchment bags to prevent accidental cross pollination.

Transmission of the monosomes in progeny was studied in selfed or outcrossed progenies of monosomic plants. Identification of the monosomes was carried out using the translocation test. For this purpose, the homozygous translocation lines were crossed as males with monosomics. Hybrids between them were analyzed to identify 2n-1 translocation heterozygotes.

Seeds of selfed or outcrossed monosomic plants and hybrids between the monosomics and the translocation lines were germinated on moist filter paper in glasses at 28 °C and after that were planted in plastic glasses with soil in a greenhouse. All seedlings were transplanted to soil of the greenhouse when the first true leaves emerged and were irrigated immediately after transplanting.

For studies of chromosome pairing at metaphase-1 (M1) of meiosis the flower buds were fixed overnight in a solution of 96 % of alcohol and acetic acid (7:3). Buds were kept at room temperature for 3 d, immersed in fresh fixative and stored in a refrigerator. For cytological preparations buds were rinsed in tap water. Then samples were examined for meiotic associations in pollen mother cells (PMCs) following iron acetocarmine squash technique. All cytological observations were carried out with the microscope *Biomed* (*Leica*, Heerbrugg, Switzerland).

## Results

A total of 84 primary monosomics were obtained between 1987 and 2000 in National University of Uzbekistan. In previous paper we pointed out on possibility to create a full series of cotton monosomics using progenies of the desynaptic disomics and monosomics (Sanamyan *et al.* 2000b).

Data of pairing of the chromosomes in meiosis for three desynaptic plants and of the monosomics recovered in their progenies are given in Table 1. The initial desynaptic plants are distinguished in number of univalents at M1 in meiosis. The first desynaptic plant ($1609/6_6$) was obtained in the progeny of plant pollinated with irradiated pollen. Chromosome association frequency per cell was 4.77I+23.61II. Second desynaptic plant ($1063/6_3$-13) was derived from the progenies of the monosomic plant without desynaptic effect. Its chromosome configuration at M1 was 14.14I+18.93II. The number of univalents ranged from 2 to 12 in the

Table 1. Chromosome pairing at meiotic metaphase 1 in the desynaptic plants and monosomic derivatives in the their progenies. The range of each configuration is given in parenthesis. Note: $1609/6_6$. $1063/6_3$-13 and 356/8 are original numbers of the disomic desynaptic plants.

| Plant number | Total number of cells | Number of chromosomes | Chromosome associations univalents | bivalents |
|---|---|---|---|---|
| $1609/6_6$ | 60 | 52 | 4.77 ± 0.48 (2-12) | 23.61 ± 0.24 (20-26) |
| Mo55 | 22 | 51 | 1.45 ± 0.18 (1-3) | 24.77 ± 0.09 (24-25) |
| Mo69 | 33 | 51 | 1.00 (1) | 25.00 (25) |
| $1063/6_3$-13 | 42 | 52 | 14.14 ± 0.97 (2-28) | 18.93 ± 0.48 (12-25) |
| Mo70 | 25 | 51 | 1.08 ± 0.08 (1-3) | 24.96 ± 0.04 (24-25) |
| Mo71 | 24 | 51 | 1.00 (1) | 25.00 (25) |
| Mo72 | 21 | 51 | 1.00 (1) | 25.00 (25) |
| Mo73 | 30 | 51 | 1.47 ± 0.15 (1-3) | 24.77 ± 0.08 (24-25) |
| 356/8 | 18 | 52 | 1.67 ± 0.18 (2) | 25.17 ± 0.09 (25-26) |
| Mo58 | 33 | 51 | 1.12 ± 0.08 (1-3) | 24.94 ± 0.04 (24-25) |
| Mo59 | 24 | 51 | 1.00 (1) | 25.00 (25) |
| Mo60 | 22 | 51 | 1.00 (1) | 25.00 (25) |
| Mo79 | 22 | 51 | 1.00 (1) | 25.00 (25) |
| Mo80 | 20 | 51 | 1.00 (1) | 25.00 (25) |

Table 2. Transmission of the monosomes in progeny in cotton monosomics ($^x$ - monosomic progeny is studied incompletely).

| Monosomic | Total plants | Disomics (26II) | Monotelodisomics (25II+1t) | Monosomics (25II+1I) | Transmission [%] |
|---|---|---|---|---|---|
| Mo2 | 6 | 5 | 0 | 1 | 16.67 |
| Mo3$^x$ | 29 | 23 | 0 | 1 | 4.17 |
| Mo7 | 12 | 9 | 0 | 3 | 25.00 |
| Mo10 | 7 | 6 | 0 | 1 | 14.29 |
| Mo11 | 9 | 6 | 0 | 3 | 33.33 |
| Mo13$^x$ | 27 | 18 | 0 | 3 | 14.29 |
| Mo15$^x$ | 45 | 32 | 0 | 1 | 3.03 |
| Mo16 | 12 | 7 | 0 | 5 | 41.67 |
| Mo17$^x$ | 20 | 14 | 0 | 3 | 17.65 |
| Mo 19$^x$ | 27 | 16 | 1 | 4 | 19.05 |
| Mo27 | 9 | 7 | 0 | 2 | 22.22 |
| Mo28 | 5 | 4 | 0 | 1 | 20.00 |
| Mo31 | 19 | 13 | 0 | 6 | 31.58 |
| Mo35 | 5 | 4 | 0 | 1 | 20.00 |
| Mo38 | 17 | 14 | 0 | 3 | 17.65 |
| Mo48 | 7 | 6 | 0 | 1 | 14.29 |
| Mo50 | 19 | 16 | 0 | 3 | 15.79 |
| Mo56 | 20 | 19 | 0 | 1 | 5.00 |
| Mo60 | 9 | 7 | 0 | 2 | 22.22 |
| Mo63$^x$ | 19 | 13 | 0 | 2 | 15.38 |
| Mo66 | 31 | 20 | 0 | 11 | 35.48 |
| Mo67$^x$ | 10 | 5 | 0 | 1 | 16.67 |
| Mo69$^x$ | 18 | 10 | 0 | 4 | 28.57 |
| Mo71$^x$ | 20 | 12 | 0 | 6 | 33.33 |
| Mo72 | 23 | 16 | 0 | 7 | 30.43 |
| Mo73 | 20 | 16 | 0 | 4 | 20.00 |

desynaptic plant $1609/6_6$ and from 2 to 28 in the desynaptic disomic $1063/6_3$-13. Chromosome counts showed that both desynaptic disomic plants had six monosomic derivatives in their progenies. The presence of these aneuploid plants in their progeny apparently was as result of irregular segregation of univalents, lagging one of univalents and producing of imbalanced gametes with deficiency of a chromosome. Third desynaptic disomic plant (356/8) was induced by thermal neutrons irradiation in $M_1$ generation and showed at M1 of meiosis two univalents only. We assume that some aneuploid plants obtained in progeny of the two desynaptic plants ($1609/6_6$ and $1063/6_3$-13) will be as nonhomological chromosomes of the cotton complement. On the contrary, five monosomics in progeny of the third desynaptic plant (356/8) may have the same monosome. This will be recovered on the basis of tests involving translocation lines.

The transmission of the monosomics was studied in the selfed or outcrossed progenies in greenhouse and in field. A total of 774 plants were cytologically analyzed in 59 of the progenies of monosomics of our collection, however, the monosomics were only found in the 26 monosomic plants. A significant portion of the progenies was disomic plants. In the progenies of cotton monosomics nullisomics failed to appear. The occurrence of monosomics was various. The frequency of the aneuploid progeny depends on the rate of transmission of the haplo-deficient gametes.

Different monosomics were distinguished by transmission rate (Table 2). In 23 plants the frequency of monosomics was ranged between 14.29 and 41.67 %. Three monosomics (Mo3, Mo15 and Mo56) usually occur in much lower frequencies (4.17, 3.03 and 5.00 %, respectively) and require larger populations to insure their recovery. Similarly, lower frequencies of the transmission are typical for the monosomics for 3, 9 and 16 (4 - 7 %) of the collection of USA (Endrizzi *et al.* 1985). Various transmission rates indirectly pointed out different monosomes to be specific chromosomes of cotton genome (Fig. 1). The least frequent aberrants appearing in the monosomic progenies were the plants with telo- or isochromosome. Three different monotelodisomics were obtained from the progenies of the Mo19, Mo61 and Mo68, although monosomics were generally not obtained in two ones (Mo61 and Mo68). A monotelodisomic plant showed pairing of 25 bivalents plus one heteromorphic pair in which the heteromorphic pair consisted of a telocentric chromosome plus a complete homologous chromosome. Sometimes heteromorphic pair was

occurred as two unequal univalents. The monotelodisomic chromosome pair was occured as two unequal univalents in 12.50 % from 16 M1 PMCs and in 14.29 % from 14 M1 PMCs, respectively for the monotelodisomic progenies of Mo68 and Mo61. In 23.81 % from 21 M1 PMCs the heteromorphic pair was occurred as two unequal univalents for monotelodisomic progenies of Mo19 which was approximately 10 % of more than that observed for above mentioned monotelodisomics. Their deficient chromosome arms have not yet been identified although it is assumed they have originated from misdivision of the monosomes. Our results conclusively demonstrated that the most of the monosomic chromosomes are fairly stable and misdivision of univalents in meiosis give only three monotelodisomics.

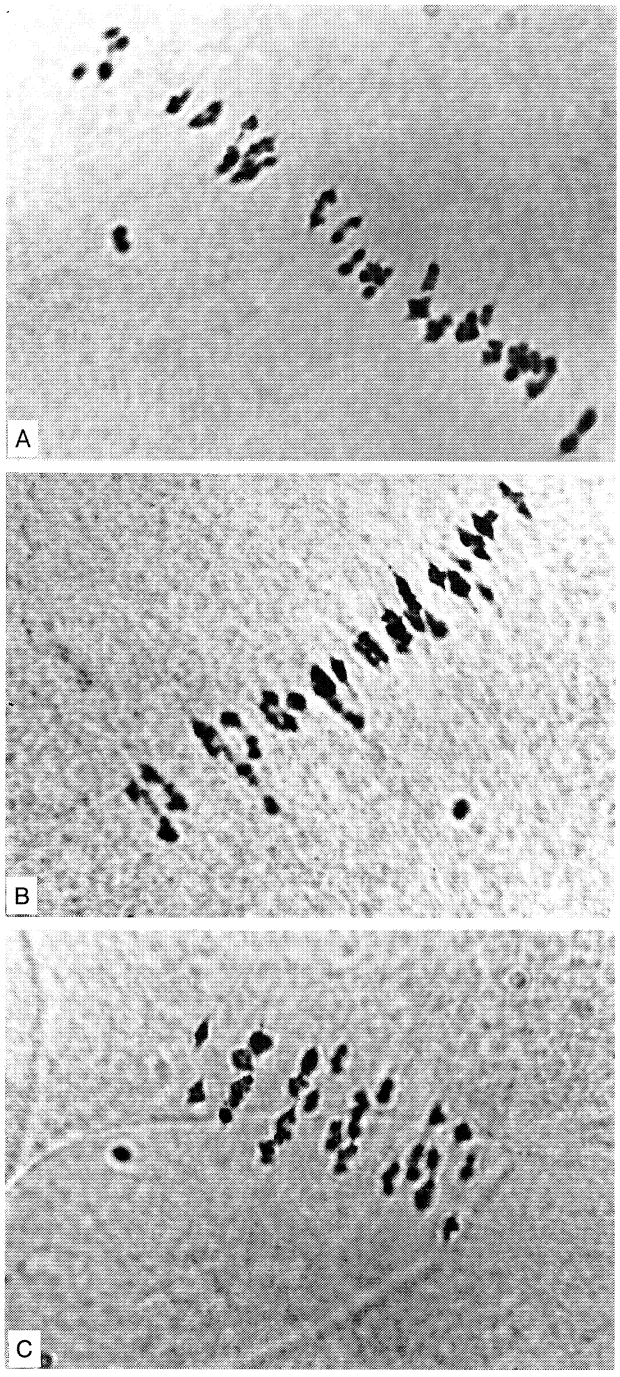


Fig. 1. Pairing of the chromosomes at metaphase-1 of meiosis in different monosomics of cotton (*A* - Mo13: $25^{II}+1^{I}$, *B* - Mo39: $25^{II}+1^{I}$, *C* - Mo66: $25^{II}+1^{I}$).

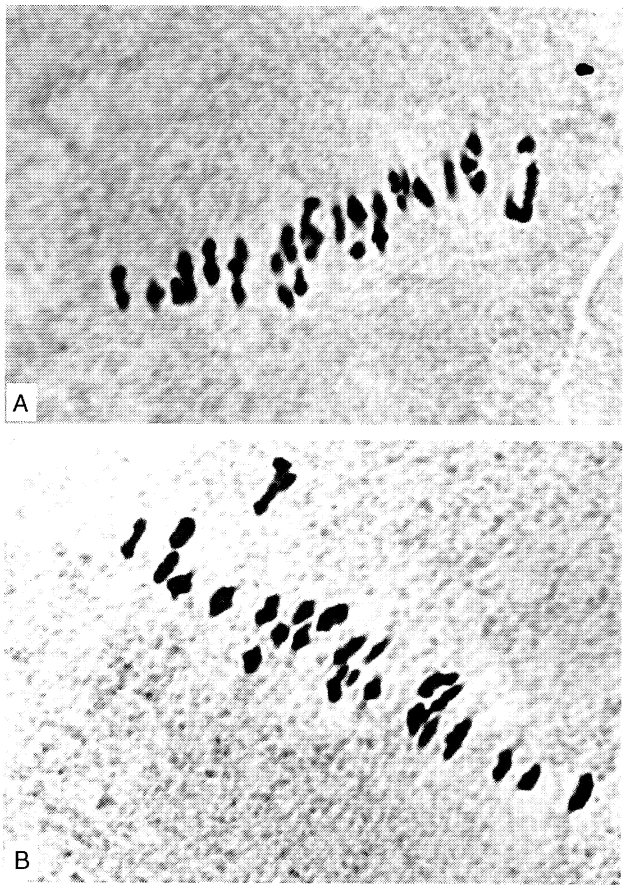


Fig. 2. Pairing of the chromosomes in critical $F_1$ plants at crosses a monosome × translocations (*A* - Mo56 × Tr6: $23^{II}+1^{I}+1^{IV}$, *B* - Mo19 × Tr16: $24^{II}+1^{III}$).

Fortunately, two monosomics were recovered from the progeny of the monosomic Mo60, which had also heterozygous translocation. The recovery of the translocation in all plants of the test-crossed progeny of first monosomic indicates that one aneuploid derivative of Mo60 is both homozygous for the medium interchange chromosome and monosomic for the medium monosome. The recovery of the monosomics from the progeny of monosomic with a translocation multivalent is surprising so far as aneuploids have not been yet recovered in the 14 progenies of the monosomics with translocations.

A number of selfed or outcrossed progenies were grown in field from 1993 to 1995. A population of 319 plants was only analyzed cytologically. However many of the progeny derivatives were not cytologically analyzed and determination of the their transmission rates were not

the primary objective. Thus it is impossible to present these results to be statistically reliable data. Cytological analyses were made on a limited number of plants from the progeny of the monosomics of the field grown the data of the investigations indicate that for the twenty progenies were determined monosomics. A significant portion aneuploids consisted of the rare monosomics which are not early determined in greenhouse (Mo22, Mo34, Mo36, Mo39, Mo40, Mo42, Mo45, Mo46, and Mo53). Furthermore, a monoisodisome was also recovered in the progeny of monosomic Mo54. It was occurred as two different univalents in 24.00 % of M1 25 cells.

Table 3. Cytological test for identification of monosomes with the help of translocation lines (+ - associated, – - independent).

| Monosomics | Translocation lines | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tr1 | Tr2 | Tr3 | Tr4 | Tr5 | Tr6 | Tr8 | Tr9 | Tr10 | Tr11 | Tr12 | Tr14 | Tr15 | Tr16 | Tr17 |
| Mo3 | | | | | – | | | | | + | – | – | | | |
| Mo7 | | – | | | – | | – | | – | | | | | – | |
| Mo10 | | | + | | – | | – | | | | | | | | |
| Mo13 | – | | | | | | | – | | | – | | | | |
| Mo19 | | | | | – | | | | | | | | | + | |
| Mo27 | | | | | | | + | | | | | | | | |
| Mo31 | – | – | | – | – | | – | | | – | – | | – | | |
| Mo36 | | – | | | | – | | | | | | | | | |
| Mo38 | | – | | – | – | | – | – | | | | | | | |
| Mo39 | | – | | | + | | – | | | | | – | | | – |
| Mo41 | | | | | | | | | | – | | | | | |
| Mo53 | | | | | | | – | | | | + | | | | |
| Mo56 | | – | | | + | – | – | | | | | | | | |
| Mo62 | – | | | | | | | | | | | | | | |
| Mo66 | – | | | | | | | | | | – | – | | – | |
| Mo69 | – | – | | – | – | | – | | | | – | | – | | |
| Mo70 | | – | | – | – | – | | – | | | | | | – | |
| Mo71 | – | | | | | – | | – | | | | | | | |
| Mo72 | – | | | – | | | | – | | | | | – | | |
| Mo73 | | | | | | | | | – | | | – | | | |

The translocation lines were used to identify monosomes (Endrizzi and Brown 1964). In the critical $F_1$ plants at crosses a monosome × translocations, a modal pairing at M1 of 23 bivalents + 1 quadrivalent + 1 univalent showed that the monosome and the chromosomes in the interchanges are different chromosomes. A modal pairing of 24 bivalents + 1 trivalent showed that the monosome is homologous to one of the chromosomes in the interchanges.

From 72 crosses between monosomics and translocation lines of our collection that involved twenty monosomics and fifteen translocation lines eight crosses are between monosomic lines and translocation line Tr2, which is multiple association involving interchange among three nonhomologous chromosomes (Table 3). None from eight monosomics (Mo7, Mo31, Mo36, Mo38, Mo39, Mo56, Mo69 and Mo70) are homologous with any of the three chromosomes in the Tr2 translocation. The monosomic plants $F_1$ had M1 pairing of 22 bivalents plus one univalent plus one hexavalent. Also, in Table 3 data are summarized for 57 crosses including monosomics - Mo7, Mo13, Mo31, Mo36, Mo38, Mo41, Mo62, Mo66, Mo69, Mo70, Mo71, Mo72 and Mo73 which are not homologous with any of the translocation lines of our collection (Fig. 2a). So as M1 pairing consisted of 23 bivalents plus one univalent plus one quadrivalent. Seven monosomics (Mo3, Mo10, Mo19, Mo27, Mo39, Mo53 and Mo56) here associated with the chromosomes of the six translocation lines (Tr11, Tr3, Tr16, Tr8, Tr5, Tr12 and Tr5, respectively). So as pairing of 24 bivalents plus one trivalent was observed in the 2n-1 $F_1$ hybrid plants (Fig. 2b).

Thus, the chromosome associations observed for the monosomes $F_1$ of Mo3 × Tr11, Mo10 × Tr3, Mo19 × Tr16, Mo27 × Tr8, Mo39 × Tr5, Mo53 × Tr12 and of Mo56 × Tr5 pointed out that these monosomes are homologous to one of the chromosomes mentioned above translocation lines. Moreover, two monosomics Mo39 and Mo56 are associated with the same translocation line - Tr5. Last may be assumed that Mo39 and Mo56 are the same monosomes of cotton genome. Furthermore, the identification may be made analysing hybrids from crosses between these monosomics and other translocation lines with chromosome in common Tr5. So

as genome tests showed that translocation lines Tr8 and Tr16 were AA-subgenome location, monosome Mo27 and Mo19 may be considered as specific A-subgenome chromosomes of the complement in cotton.

## Discussion

Complete sets of monosomics have been created in wheat (Sears 1954), oats (McGinnis 1966, Morikawa 1985) and tobacco (Clausen and Cameron 1944). American Cotton Cytogenetic Collection includes 15 monosomic lines from 26 and 29 monotelodisomics from 52 need to cover the entire cotton genome. Development of one or more deficiencies that would involve part or all of these chromosomes has therefore been high priority (Stelly 1993).

In previous article we proposed that a full series of monosomics of cotton will be probably created using the progeny of the desynaptic disomics or monosomics so far as the radiation treatment of seeds or pollen induced the losses of certain chromosomes - "traditional monosomes" (Sanamyan *et al.* 2000b). In this report we cytologically characterized eleven monosomics in cotton obtained in the progeny of the three disomic desynaptic plants. The results reported here demonstrate that desynaptic plants of cotton produced the progeny that includes a number of aneuploids which will be used for the supplement of monosomic series after their identification. However, we cannot rule out the possibility that the monosomics in the progeny of the desynaptic plants may also have A-subgenome monosomes as Myles and Endrizzi (1989) noted that the A-subgenome chromosomes undergo nondisjunction significantly more frequently that do the D-subgenome chromosomes because of more peripheral position the A-subgenome chromosomes in the nucleus.

Earlier it has been reported that aneuploids may arise in various ways, such as abnormal disjunction in meiosis of desynaptic mutants (Burnham 1962). Khush (1973) suggested that because of irregular segregation of univalents in the asynaptic genotypes imbalanced gametes are produced. When such gametes are fertilized by the *n* gametes, monosomic progeny is produced. Clausen and Cameron (1944) reported that an asynaptic genotype of *Nicotiana tabaccum* called pale-sterile was employed for isolating monosomics. One monosomic was isolated in the progeny of an asynaptic strain of *G. hirsutum* also by Brown and Endrizzi (1964). Only two monosomics in cotton were recovered from progenies of desynaptic plants in USA (Edwards *et al.* 1980). Moreover these two were mono-4 of the A-subgenome with the most frequent rate of transmission. The examination of the breeding behaviour of the monosomics during a number of years recovered monosomics only in the 26 monosomic progenies. Failure to produce monosomics from a number progenies was probably due to the small numbers of progeny which chromosomes were studied or to the low germination of the seeds. It is interesting to note that two of the monosomics of cotton failed to transmit to the next generation in USA (Brown and Endrizzi 1964). As pointed out by Endrizzi *et al.* (1985) the frequency of monosomics of cotton was ranged between 20 and 50 % except the those for chromosomes 3, 9 and 16 which usually were occurred in much lower frequencies. On the whole our results confirmed the data of American investigators and pointed out the transmission rates through the female of monosomics of cotton to be lower than those of the monosomics of wheat and tobacco (Clausen and Cameron 1944, Sears 1954, Endrizzi *et al.* 1985).

Three monotelodisomics and one monoisodisomic were only recovered from a number of the progenies of monosomic plants from more than 700 plants of cotton. It is pointed out on the high stability of cotton monosomes and on the rare misdivision of univalent chromosomes in meiosis. The fertility of pollen of these plants was rather high. The part of sterile pollen grains was due to nondisjunction of two univalents. It is known that the most pollen produced by the PMCs with two univalents are likely to be deficient for a chromosome and nonfunctional (Endrizzi and Bray 1980). According to Brown (1958) and Endrizzi *et al.* (1985) the most of the monosomic chromosomes of cotton are fairly stable and misdivide infrequently to give telocentrics or isochromosomes. Lately Endrizzi and Ramsay (1980) reported that the monosome 12 of cotton undergoes a rather high rate of misdivision and the chromosome 25 has a very high one. Unfortunately, the calculated data were not publicated about frequency of telosomes and isochromosomes of cotton as it was reported by Sears (1954) for wheat.

Since cotton chromosomes have small sizes and from median to submedian centromeres, 26 chromosomes can be distinguised cytologically according to translocation tests. Unfortunately the translocation lines from Cytogenetical Collection of USA were not available at the time of these experiments. Therefore the uniform identification of monosomics of our collection was not possible.

In this study we could determine the identity of seven monosomes and one of the interchanged chromosomes of six lines having translocations from our collection. We failed until to identify the missing chromosomes in other monosomic lines. A final conclusion cannot be drawn without further cytogenetical studies. However more accurate evaluation of the above mentioned monosomes may be carried out by genome *in situ* hybridization (FISH and GISH) and other cytogenetic molecular techniques.

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