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The development and a cytogenetic study of monosomics of *Gossypium hirsutum* L.

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Abstract

Monosomics of cotton (*Gossypium hirsutum* L.) were obtained by irradiation of pollen by γ-rays and by irradiation of seeds by thermal neutrons. Many monosomics were derived directly from irradiation, but a number of monosomics were also recovered in the progeny of plants with translocations and of desynaptic plants. Only 28 primary monosomics showed normal pairing at metaphase-1 of meiosis. The others formed rare trivalents or additional univalents. Partial desynapsis was detected in some monosomics. The pollen fertility levels of monosomics are presented. New morphological characters were detected among the monosome plants of cotton.

Additional key words: cotton, primary monosomes, univalent, desynapsis, pairing.

Introduction

Allotetraploid cotton consists of "duplicated genomes" and can tolerate the loss of whole chromosomes. Because of rare occurrence of spontaneous monosomic mutations and very low percentage of fertile female haploid plants of cotton, studies have been initiated to obtain monosomes artificially (Brown and Endrizzi 1964, Endrizzi and Brown 1964, Endrizzi 1966, Galen and Endrizzi 1968).

The majority of monosomes are for chromosomes 2, 4 and 6 of the A-subgenome (Endrizzi and Ramsay 1979, Edwards *et al.* 1980). At the present, 15 monosomes for 26 chromosomes of *G. hirsutum* were detected (Endrizzi *et al.* 1985). These identified chromosomes are 1, 2, 3, 4, 6, 7, 9, 10 and 12 for the A-subgenome and 16, 17, 18, 20, 22 and 25 for the D-subgenome.

The monosomic stocks have been used to determine genetic markers to specific chromosomes (Endrizzi 1963, Kohel and Douglas 1974, Endrizzi and Stein 1975, Endrizzi and Bray 1980, Endrizzi and Ramsay 1980, 1983, Stelly 1990, Endrizzi and Ray 1991, 1992, Samora *et al.* 1994). The Cotton Cytogenetic Collection, includes 58 simple reciprocal translocations breakpoints of which involve 25 from 26 chromosomes and 43 from 52 of chromosome arms and 15 monosomic and 29 monotelodisomic upland types (Stelly 1993). The monosomic and monotelodisomic stocks allow to determine 19 linkage groups in 26 chromosomes, and 34 linkage groups in 52 chromosome arms.

One of the main uses of monosomes is the determination of loci of marker genes on specific chromosomes. The first chromosomal assignment in cotton - the biochemical marker locus, specifically phosphoglucomutase 7 (Pgm 7) was reported by Saha and Stelly (1994). During last 15 years, our investigations have been directed towards creation of series of lines with the chromosomal markers (Sanamyan and Musaev 1990, 1992, 1995). The aim of the present paper is to give basic information on the origin, chromosome pairing at metaphase-1 of meiosis, sizes of univalents, fertility of pollen and morphological characters of the monosomics in cotton induced and discribed at the Tashkent State University.

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Materials and methods

Inbred line-L-458 of cotton (*Gossypium hirsutum* L.) from genetic collection of Tashkent State University was used. This line was selected from a commercial cultivar 108-F.

A day before flowering, the flowers were emasculated and enclosed in parchment bags to prevent accidental cross pollination. On the day of flowering the flowers with mature pollen were collected and irradiated with 10, 15, 20 and 25 Gy γ-radiation (Co^{60}, Central Asiatic Institute of Silk, Tashkent), and then used to pollinate the emasculated flowers. All seeds obtained in this study were M_0 generation. In another experimental set, thermal neutron irradiation of L-458 seeds was carried out at the biological channel reactor *VVR-SM* (Institute of Nuclear Physics of Academy Science of Republic of Uzbekistan, Tashkent). Doses of 15, 25, 27 and 35 Gy were used. Next year seeds M_0 were grown in field. The plants M_1 were cytogenetically studied and were used to raise M_2 generation. The M_2 plants were grown in field and cytogenetically investigated for chromosome aberrations.

For studies of chromosome pairing at metaphase-I of meiosis the flower buds were fixed overnight after removing of calyx and corolla 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 association in pollen mother cells (PMCs) following iron acetocarmine squash technique. Pollen fertility was estimated by acetocarmine staining. Meiotic index was calculated as percentage of normal tetrads.

Results and discussion

In our experiments, 73 monosomics (Mo1 - Mo73) of cotton were obtained by γ-irradiation of pollen and by irradiation of seeds by thermal neutrons. Numbers of monosomics obtained differed in M_1 and M_2 generations and after treatment by different doses of irradiation (Table 1). So, γ-irradiation of pollen revealed in the first generations (M_1) 34 plants with deficient chromosomes, and 7 of them had also interchanges. During studies of the M_2 generation of the changed plants, further 23 primary monosomics were detected, and two of them had translocations. Six monosomics were also derived in the progeny of the disomic plants with desynaptic genotype. Treatment of seeds by thermal neutrons induced 10 primary monosomics in M_1 and M_2 generations, one monosomic had an interchange.

It is necessary to note, that many of the monosomics were derived directly from irradiated materials, but a number of monosomes were also recovered in the progenies of plants with translocations and of a desynaptic plant. For example, Mo30 and Mo67 monosomics were found in the progenies of the plants with translocations, desynaptic genotypes or two unpaired chromosomes. It was a case of irregular segregation of two univalents and so imbalanced gametes were produced. As the result the monosomics Mo30 and Mo67 were recorded. Monosomics Mo55 and Mo69 were found in the progeny of a desynaptic plant which formed at metaphase-I two to twelve univalents. Moreover, monosomics Mo70 - Mo73 were obtained in the progeny of a desynaptic disomic plant, in which many univalents (from 2 to 28) were present at metaphase-1 of meiosis. Apparently, the origin of these monosomic plants resulted from irregular segregation. The isolation of the monosomic from an asynaptic strain *G. hirsutum*

Table 1. The origin of primary monosomes of cotton.

Treatment	Dose [Gy]	Number of plants M_1	M_2	Number of monosomics M_1	M_2
γ-irradiation of pollen	10	46	58	5	4
	15	52	55	4	9
	20	87	72	11	8
	25	66	36	14	2
Irradiation of seeds by thermal neutrons	15	15	51	-	4
	25	19	23	-	1
	27	20	58	-	1
	35	21	53	2	2

has been reported by Brown and Endrizzi (1964). An asynaptic genotype of *Nicotiana tabacum* called palesterile was also employed for isolating monosomics (Clausen and Cameron 1944).

It is interesting to note that the monosomic Mo25 was found in the progeny of a plant with unknown karyotype, but with known low meiotic index and with high frequency of microspore's tetrads with micronuclei. It is known, that the proportion of n and n-1 spores produced by monosomics depends on the frequency of lagging and misdivision of the univalent. Estimation of the proportion of n and n-1 spores may be obtained by determining the frequency of spore tetrads with micronuclei (Khush 1973). Monosomic Mo25 was probably derived from microspore tetrads with micronuclei as a result of lagging or misdivision of the univalents.

Little is known about the exact mechanism by which radiation treatment induced monosomy. It has been shown early, that if the chromosome is broken or damaged in the centromere region it is impossible to show normal centromeric activity (Khush 1973). Since *G. hirsutum* is an allotetraploid, it is possible to obtain reproductive forms that are deficient for one or more chromosomes.

Cytological analyses of the meiotic behaviour of chromosomes of cotton monosomics show normal pairing with 25 bivalents plus one univalent at the metaphase-1 of meiosis. In our experiments, only 28 primary monosomics showed such normal pairing. Five monosomics Mo6, Mo7, Mo30, Mo56 and Mo 62 formed a rare trivalent when the univalent chromosome paired with two homoeologous chromosomes. The additional univalents (from 3 to 5) observed in certain cells in some monosomics might be due to early separation of one or three homologous bivalents or due to the non-conjunctional behaviour of one or three pairs. Occurrence of more than one univalent in monosomic *Nicotiana tabacum* has also been reported (Clausen and Cameron 1944).

Strongly varying numbers of univalents at metaphase-1 were noticed in some monosomics of cotton (Table 2). Thus Mo17 and Mo47 monosomics had three univalents in the first metaphase of meiosis (42.86 and 27 %, respectively) in most of PMCs. Monosomic Mo17 showed also albino phenotype. In is possible to suppose that Mo17 and Mono-1 from the American collection involved the same chromosomes from A-subgenome.

The partial desynapsis were characterised by few to many univalents (Mo43, Mo44, Mo45 and Mo48). Two monosomics (Mo49 and Mo52) were strongly different from others by forming a large number of additional univalents. Thus in Mo49, the number of univalents formed in the metaphase-1 in PMCs ranged from 1 to 11 and only in half of PMCs normal pairing was determined. Mo-52 had not normal pairing with 25 bivalents plus one univalent at the metaphase-1 of meiosis. The number of univalents formed at the metaphase-1 ranged from 3 to 15 (in average 8.00 ± 0.88 per cell) in all PMCs studied. Irregular segregation during subsequent stages of meiosis resulted in the formation of microspore tetrads with micronuclei and abnormal pollen grains.

It is known, that some monosomics, partially asynaptic due to the imbalanced chromosome number, constantly produce monosomics for different chromosomes in their progenies. Sears (1954) had isolated monosomics for 17 different chromosomes in the progenies of partially asynaptic nulli-3B of *Triticum aestivum*.

Monosomics Mo49 and Mo52 were obtained in M_2 generation after irradiation of pollen. We assume that these monosomics are characterised by the loss of a chromosome and by the mutation of desynaptic genes. This hypothesis could be confirmed by cytological research of next generation of monosomics Mo49 and Mo52 if it is possible to obtain disomic desynaptic plants of cotton.

It is known, that reduction of pairing may be caused by genes with the specific effect on chromosome pairing in cotton (Brown and Menzel 1953). The inheritance of the asynapsis which was controlled by two loci with the recessive allels in homozygous condition was demonstrated by Beasley and Brown (1942).

Table 2. Chromosome associations at M-1 of meiosis observed in PMCs of monosomics of cotton (pollen irradiation). Means ± SE. n = 26.

Material	Dose [Gy]	Number of cells	Number of chromosome associations univalents	bivalents	Frequency of chromosome associations (average per cells) univalents	bivalents
Mo-17	25	35	1-3	25-24	1.86 ± 0.17	24.57 ± 0.08
Mo-43	20	21	1-5	25-23	2.14 ± 0.33	24.43 ± 0.16
Mo-44	25	20	1-7	25-22	2.80 ± 0.43	24.10 ± 0.21
Mo-45	25	26	1-7	25-22	2.85 ± 0.37	24.08 ± 0.18
Mo-47	25	52	1-3	25-24	1.54 ± 0.12	24.73 ± 0.06
Mo-48	25	30	1-5	25-23	1.93 ± 0.23	24.53 ± 0.07
Mo-49	10	32	1-11	25-20	3.38 ± 0.58	23.81 ± 0.29
Mo-52	15	24	3-15	24-18	8.00 ± 0.88	21.50 ± 0.44

The allotetraploid species *G. hirsutum* contains two subgenomes: A-subgenome with large chromosomes and D-subgenome with small chromosomes. Preliminary, six of our monosomics have small univalent chromosomes, in the others univalent chromosomes were large or medium. Full identification of monosomics will be done after genomic and translocation analyses with the help of interspecies hybrids F_1 of *G. thurberi* × *G. raimondii* and homozygous translocations lines from our collection (Musaev *et al.* 1997).

A-subgenome chromosomes are recovering as monosomes not only more frequently than D-subgenome chromosomes, but at least two of A-subgenome chromosomes are recovered with more than random frequency. *G. hirsutum* can tolerate the loss or addition of large chromosomes without great effect on vigour or fertility (Brown and Endrizzi 1964).

The number of monosomic plants isolated in M_1 were characterised by the decreasing of meiotic index and by the increasing of percentage of tetrads with micronuclei. The monosomic plants Mo5, Mo20, Mo44 and Mo65 were sterile. Pollination by normal pollen did not help to induce seed setting. The pollen fertility of monosomics from our collection was significantly different and varied from high fertility (93 - 97 %) for Mo4, Mo29, Mo40, Mo62; semisterile (45 - 67 %) for Mo3, Mo31, Mo33, Mo38, Mo41, Mo61, Mo67, Mo68 to low fertility (2 - 34 %). In 8 monosomics the percentage of fertility of pollen varied between flowers on the same cotton plant. In whole, the high frequency of aborted pollen grains in flowers was typical for many monosomics of cotton.

Many of cotton monosomics had typical morphological characteristics: slow growth, thin stems, short sympodia, small leaves and bolls, decreased pollen shedding.

Among the monosomic plants of our collection new morphological markers were detected, not detected in the Cytogenetical collection in USA (Endrizzi *et al.* 1985). Such markers are: ribbed flattened bolls (Mo7), spherical bolls with shark beaks (Mo48), dense hairiness (Mo13, Mo34), heavy flowers and bolls (Mo 15), gigantism (Mo39), and "iron tree" with hard inflexible stem (Mo45, Mo47).

The data presented in this article strongly suggest that the creation of monosomic plants of cotton is a very difficult problem. During long-term studies in USA full series of monosomics of cotton were not obtained due to the rare occurrence of spontaneous monosomics and a complete sterility of haploids. Moreover, a vast majority of induced monosomics were from A-subgenome (Endrizzi *et al.* 1985). The reason that further monosomics were not recovered might be the absence of variability and transmission in generation, or normal phenotype and similarities with identified monosomics or that they never had been encountered (Menzel 1982). In our opinion, the radiation treatment of seeds or pollen induces the losses of certain chromosomes - "traditional monosomes" only. In future, it is possible to create a full collection of monosomics using the progeny of the desynaptic plants or desynaptic monosomics.

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