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The Utilization of Translocation Lines and Microsatellite Markers for the Identification of Unknown Cotton Monosomic Lines

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Additional information is available at the end of the chapter

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Abstract

Simple sequence repeats (SSR) have been applied as useful markers for understanding cotton genetics. In the last decade, chromosome-deficient stocks of *Gossypium hirsutum* L. were used in the development of chromosome substitution lines for *G. barbadense* L., *G. tomentosum* Nuttall et Seemann, and *G. mustelinum* Watt chromosomes or chromosome segments. Several DNA markers have already been assigned to the individual chromosomes of *G. hirsutum*. We created new cotton monosomic lines in Uzbekistan after irradiation of seeds by thermal neutrons or pollen gamma-irradiation to complement other global efforts in the development of cotton chromosome substitution lines. The primary objective of this chapter is to report the use of chromosome-specific SSR markers and a well-defined tester set of cotton translocation lines from the Cotton Cytogenetic Collection at Texas A&M AgriLife Research to confirm chromosome specificity of monosomic lines in Uzbekistan cytogenetic collection of cotton. Our results have assigned several different monosomic lines to the chromosomes 2, 4, 6, and telosome 11 A_t-subgenome and chromosomes 18 and 20 or 22 D_t-subgenome. These lines will be very useful in molecular mapping, the creation of substitution lines, and cotton breeding.

Keywords: cotton (*Gossypium hirsutum* L.), chromosome, identification, monosome, molecular markers

1. Introduction

Monosomic plants can be used for the synthesis of the chromosome substitution (CS-B) lines [1]. Such substitution lines are a useful means of interspecific introgression and for studying economically important genes. In upland cotton (*Gossypium hirsutum* L.), Kohel et al. [2] studied multiple traits of disomic substitution lines that contained chromosomes 6 or 17 of *G. barbadense* (L.).

About 10 years ago, replacement and additional *G. barbadense* chromosome substitution lines became available. These were developed similarly by hybridization of the same *G. barbadense* donor line to quasi-isogenic *G. hirsutum* hypoaneuploids, followed by iterative modified backcrossing and then inbreeding. Each resultant CS-B line was expected to be substituted for one chromosome or segment and to be largely though not necessarily completely devoid of unrelated *G. barbadense* segments [3, 4]. The results revealed information on the association of specific chromosomes with genes for agronomic and fiber traits. More recently, substitution lines were created for *G. tomentosum* [5] and *G. mustelinum* [6], as well as for additional chromosomes of *G. barbadense* [7]. Saha et al. subsequently used the CS-B lines to identify chromosomal locations of important traits and beneficial genes and interactions [3, 8, 9].

Simple sequence repeats (SSR) of cotton have been applied widely as molecular markers for genetics, mapping, trait analysis, and germplasm diversity analysis and comparisons of specific individuals, lines, and populations. Several SSR markers associated with lint percentage have been located to chromosomes 12, 18, 23, and 26 using deletion analysis in aneuploid chromosome substitution lines. SSRs were used to analyze the genetic identities of specific hypoaneuploid interspecific F_1 hybrids and disomic chromosome substitution intermediates genotypes, using the principles of deletion molecular analysis [10, 11]. Recent reports extended the systematic characterizations of additional hypoaneuploid chromosome substitution F_1 hybrids and many chromosome substitution lines using chromosome-specific SSR-markers [6, 7].

A Cotton Cytogenetic Collection of Uzbekistan now includes 95 primary monosomics derived from the highly inbred *G. hirsutum* line "L-458" by irradiation of the seeds with thermal neutrons and pollen with gamma-rays [12]. The chromosome identities of these monosomic lines of the Uzbek Collection need to be determined to optimize the efficiency of collection maintenance and its use in breeding-related research, for example, development of chromosome substitution lines.

The primary objective of this chapter is to report the use of chromosome-specific SSR markers and a well-defined tester set of translocation lines of the USA collection to confirm chromosome specificity of monosomic lines in Uzbekistan collection.

2. Materials and methods

2.1. Plant material

Monosomic lines of the Uzbek Cytogenetic collection were developed in a common genetic background of the highly inbred line *G. hirsutum* line L-458, which was created through

multiple generations of self-pollination (F_{20}) of cultivar 108-F [13, 14]. Because all of the 95 primary monosomics were isolated from a common genetic background, some differences observed among them can be attributed to the differences in their monosomic state [15]. Irradiation of seeds by thermal neutrons or pollen gamma-irradiation gave rise to most (76) of the monosomics. Other monosomic plants were detected among progenies of desynaptic plants (17) and translocation heterozygote plants (2). The primary monosomics were numbered according to their order of detection (Mo1-Mo95).

The *G. barbadense* line 3-79 is nonphotoperiod sensitive and is highly homozygous as it is originated as a doubled haploid [16]. It has been used extensively as parent in genetic studies, genomics [17], and as a donor parent for the substituted chromosome (CS) or chromosome segments from *G. barbadense* in our study. The overall method of the CS line development was discussed in previous reports [4, 18].

A well-defined tester set of translocation lines of Cytogenetic Collection of the USA was kindly provided by Prof. D.M. Stelly through a USDA-Uzbekistan cotton germplasm exchange program. All plant materials were vegetatively maintained in the greenhouse of National University of Uzbekistan.

2.2. Cytological analyses

For studies of meiotic chromosome pairing at metaphase-I (MI) and sporad normality floral buds were collected in the morning, and after the removal of calyx and corolla, fixed in a solution of 96% alcohol and acetic acid (7:3). Buds were kept at room temperature for 3 days then immersed in fresh fixative and stored at 4°C. For cytological preparations, buds were rinsed in tap water before being examined for meiotic MI chromosome configurations in the microsporocytes, commonly known as “pollen mother cells” (PMCs) using the iron acetocarmine squash technique. Analyses of hybrid plant chromosomes were carried out on the basis of MI configurations. The development of F_1 hybrid plant PMCs was assessed based on the cytological features observed at the tetrad stage. The meiotic index was calculated as the percentage of sporads that were normal tetrads. Pollen viability was estimated as percentage of mature pollen grains stainable in acetocarmine. All cytological observations were made using Biomed (Leica, Heerbrugg, Switzerland) and Axioskop A1 (Carl Zeiss, Germany) microscopes.

2.3. Identification of hybrid F_1 monosomic translocation heterozygotes

Monosomes were chromosomally identified using translocation tests. For this purpose, the monosomic ($2n = 51$) lines from Uzbek Cytogenetic Collection were crossed as seed parent with translocation lines ($2n = 52$) of the tester set from USA Cotton Cytogenetic Collection provided by Texas A&M AgriLife Research. Floral buds of F_1 hybrid progeny were analyzed to identify individuals that were monosomic ($2n = 51$) and also heterozygous for the respective translocation, that is, monosomic translocation heterozygotes. To reveal “critical configurations” and detect common chromosomes among the chromosomes involved in interchanges with monosomes, the meiotic MI configurations were analyzed in heterozygotes of monosomic

translocations heterozygous F_1 hybrids. Progeny modally forming 23II + IV + I were interpreted as indicating independence between chromosomes affected by monosomy and the translocation, whereas progeny modally forming 24II + III were interpreted as evidence of association between the monosome and one of the two translocation chromosomes. It was generally presumed that parent and progeny monosomic conditions were similar, that is, no monosomic “shift” occurred.

2.4. DNA extraction and genotyping

Genomic DNAs were extracted from young leaf samples of cytogenetically identified F_1 monosomic cotton hybrids using CTAB method [18]. Extracted genomic DNAs were checked in 0.9% agarose electrophoresis, and DNA concentrations were diluted to a working concentration of 15 μ l based on Hind III-digested λ -phage DNA 25 ng/ μ l. PCR amplification carried out in a 10 μ l volume reaction mix containing 1.0 μ l 10 \times PCR buffer, (with 25 mM Mg Cl₂), 0.2 μ l BSA, 0.08 μ l dNTPs (25mM), 0.2 μ l primer pairs, 0.1 μ l Taq-polymerase, and 2 μ l DNA template. PCR runs were conducted with an initial denaturation of DNA at 94°C for 2 min, followed by 35 cycles of 94°C (step 1) for 20 sec, 55°C (step 2) for 30 sec, and 72°C (step 3) for 50 sec. After 35 cycles, the extension temperature of 72°C was held for 7 min. The PCR products were visualized in 3.5% high-resolution agarose gel, stained with ethidium bromide and photodocumented using an Alpha Imager (Innotech Inc., USA) gel documentation system.

Chromosome-specific SSR primer pairs were collected according to recently published genetic mapping papers [11, 19–27]. For each chromosome, we chose four loci that were polymorphic between L-458 (*G. hirsutum*) and 3-79 (*G. barbadense*). Documented electrophoregram results for SSRs were scored as “a/b/h” where “a” locus similar to recipient L-458, “b” locus similar to donor line 3-79 and “h” genotype similar to normal F_1 hybrid. To identify the chromosome deficient from a given F_1 monosomic cotton hybrid, the SSRs of that chromosome were expected to exhibit “b” genotype, that is, to lack of the *G. hirsutum* (maternal) allele and to possess only the *G. barbadense* (paternal) allele [10].

3. Results

3.1. Cytogenetic characteristics of the aneuploid hybrid F_1 plants

We crossed 46 monosomic and two monotelodisomic lines of *G. hirsutum* from Uzbek cytogenetic collection of cotton and a *G. barbadense* doubled haploid line Pima 3-79. Among resulting progeny, the aneuploid hybrid F_1 plants were detected in 37 interspecific F_1 progeny families based on hybrid phenotypes and meiotic metaphase-I configuration analyses. In five of the aneuploid hybrid F_1 families, three hybrid monosomic plants were detected in each progeny, in 11 aneuploid hybrid F_1 families two hybrid monosomic were isolated, and in the remaining 21 hybrid families, one aneuploid hybrid F_1 plant was detected. These results showed differences in the ease monosomic detection in various hybrid backgrounds and/or differences in maternal transmission rates for various monosomes. Deficiencies for one chromosome arm occurred in the progenies of two monotelodisomic hybrid F_1 plants.

Hybrid F_1 plants had intermediate phenotypes, including strong effects on plant shape and size; clustering (flower positions and density); number, sizes and shapes of leaves, bracts, and stipules; sizes and number bract teeth; coloration; number and sizes of external nectarines; and boll shape. Additional phenotypic effects by monosomy were super-imposed on these general phenotypic effects of interspecific hybridity.

Meiotic metaphase-I analysis of 56 monosomic hybrid F_1 plants revealed that 46 plants exhibited modal chromosome pairing of 25 bivalents and one univalent. Seven monosomic hybrid F_1 plants were characterized with the presence of additional univalents and their average univalent frequencies per cell among them ranged from 1.06 ± 0.06 in Mo67xPima 3-79 to 1.22 ± 0.15 in Mo11xPima 3-79. Three univalents were observed in some PMCs of the seven monosomic hybrid F_1 plants from Mo7, Mo11, Mo27, Mo67, Mo72, Mo75 and Mo89. Three monosomic hybrid F_1 plants from families Mo17xPima 3-79 formed three univalents in many PMCs, and they accordingly exhibited relatively high average univalent frequencies per cell, which ranged from 1.24 ± 0.13 to 1.45 ± 0.15 . The similarity of relative univalent sizes across the affected cells (24 II + 3 I) suggested that the extra pair of univalents in all of these cells arose from the same pair of chromosomes. A similar effect was noted in the parental monosomic plant in selfed Mo17 progenies, and could indicate that Mo17 is affected by more than simple monosomy. The three monosomic hybrid F_1 plants (Mo17xPima 3-79) also seemed to differ in their morphology, which suggests the possibility that one or more of them arose following a univalent shifts due to irregular chromosome disjunction in meiosis of the maternal Mo17 parent.

Meiotic products were characterized by examining sporads in most of the monosomic hybrid families, looking for example for the frequency of normal tetrad conformation. Meiotic index, originally proposed by Love [28] for evaluation of meiosis in wheat, reports the normal tetrad percentage and is an indicator of meiotic stability. Most of monosomic hybrid F_1 plants had a higher meiotic index (more than 90%) than that of the control hybrid F_1 plants (L-458xPima 3-79— $89.25 \pm 0.58\%$), which indicated that their univalent chromosome underwent regular disjunction. However, the meiotic index values of four of the monosomic hybrid F_1 plants (Mo48xPima 3-79, Mo59xPima 3-79, Mo62xPima 3-79, Mo92xPima 3-79) were characterized ranging from $89.78 \pm 0.50\%$ to $85.96 \pm 1.29\%$. Two monosomic hybrid F_1 plants showed an increase of percentage of tetrads with micronuclei to $4.41 \pm 0.45\%$ (Mo59xPima 3-79) and $4.53 \pm 0.37\%$ (Mo92xPima 3-79) in comparison with control hybrid F_1 plants (L-458xPima 3-79— $1.02 \pm 0.06\%$), which demonstrated disturbances in monosome disjunction and formation of imbalanced gametes.

Pollen viability after acetocarmine staining was studied in monosomic hybrid families. Pollen viability was high in most monosomic hybrid F_1 plants. However, nine monosomic hybrid F_1 plants had reduced pollen viability and showed early haplo-deficient microspore abortion prior to mature pollen stage. Two monosomic hybrid F_1 plants had small reductions in pollen viability (up to 20%), four monosomic hybrid F_1 plants had more reductions in pollen viability (up to 30%), and three monosomic hybrid F_1 plants had reductions in semisterile pollen (up to 50%). Thus, some of monosomic hybrid F_1 plants had decreased meiotic index and decreased pollen viability.

3.2. Molecular marker analysis

For molecular analysis of the monosomic hybrid F_1 plants, the principles of deletion molecular analysis were used [10, 11]. Considering that many DNA markers have already been assigned to the individual chromosomes of *G. hirsutum* L., we aimed to utilize chromosome-specific SSR markers to identify and reconfirm the chromosome specificity identities of monosomic lines of our collection, based on SSR content monosomic substitution F_1 hybrids created by crossing with the doubled haploid *G. barbadense* L. line 3-79. Detection and genotyping of SSR markers were straightforward and in a manner described in previous reports that utilized a PCR amplification of chromosome-specific markers in the genomic DNAs of hybrid plants. To localize SSR loci to chromosomes, we screened monosomic hybrid F_1 plants for the L-458 allele using labeled and/or unlabeled primers. For SSR loci located at sites other than the chromatin deficient segment, the L-458 marker would be present and F_1 hybrids would exhibit heterozygous phenotype. In comparison, if an SSR locus was on the segment deficient from the hypoaneuploid hybrid F_1 plant, the electropherogram would lack the L-458 allele and exhibit hemizygous pattern for the donor allele from *G. barbadense* Pima 3-79.

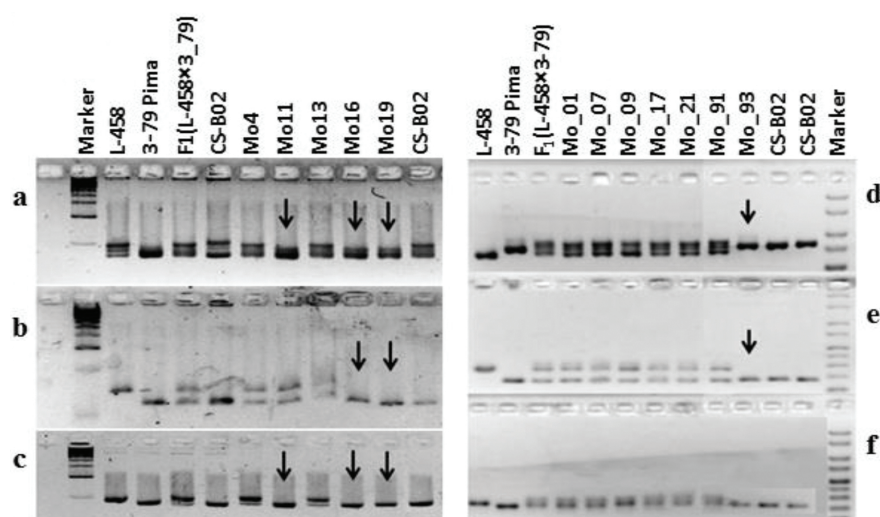


Figure 1. Chromosome identification of monosomic interspecific F_1 hybrids, monosomic *G. hirsutum* × *G. barbadense*. SSR primer pairs specific for chromosome 2 A_1 -subgenome: (a) BNL3590; (b) BNL3971; (c) Gh198; (d) BNL1434; (e) BNL3971; and (f) BNL1897.

Our results revealed that four monosomic hybrid F_1 plants (Mo11xPima 3-79, Mo16xPima 3-79, Mo19xPima 3-79, and Mo93xPima 3-79) deficient for unknown chromosome(s) showed the presence of only *G. barbadense*-specific SSR markers bands (BNL3590 and GH-198 for Mo11), (BNL3971, BNL3590, and GH-198 for Mo16 and Mo19), (BNL1434, BNL1897, and BNL3971 for Mo93), and corresponding absence of the respective L-458 allele. The results helped reveal the chromosomal identities of monosomes Mo11, Mo16, Mo19, and Mo93 based on known chromosomal locations of the respective SSR markers. Because the aforementioned SSR markers were assigned previously to chromosome 2 of the A_1 -subgenome of cotton [11, 19–

27] the SSR-based results for Mo11, Mo16, Mo19, and Mo93 indicate that they are monosomic for cotton chromosome 2 (**Figure 1**).

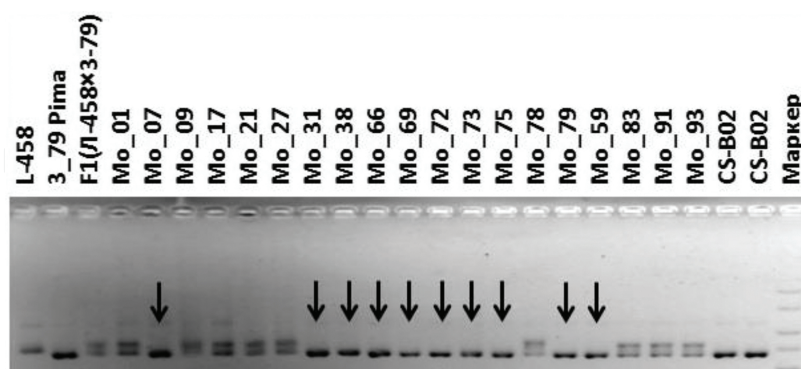


Figure 2. Chromosome identification of monosomic interspecific F_1 hybrids, monosomic *G. hirsutum* \times *G. barbadense*. SSR primer pairs specific for chromosome 4 A_1 -subgenome: BNL2572.

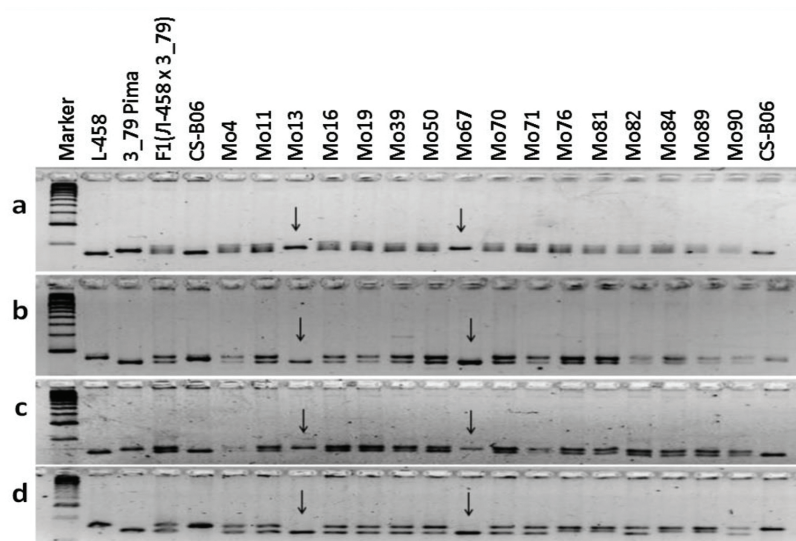


Figure 3. Chromosome identification of monosomic interspecific F_1 hybrids, monosomic *G. hirsutum* \times *G. barbadense*. SSR primer pairs specific for chromosome 6 A_1 -subgenome: (a) BNL2884; (b) CIR203; (c) BNL1064; and (d) Gh082.

SSR-based deficiency analysis of the six monosomic hybrid F_1 plants (Mo70xPima 3-79, Mo71xPima 3-79, Mo76xPima 3-79, Mo81xPima 3-79, Mo89xPima 3-79, and Mo90xPima 3-79), each deficient for an unknown chromosome showed the presence of only *G. barbadense*-specific SSR marker bands for BNL2572, CIR122, and GH-107, was lacking the respective L-458 alleles. Similarly, analysis of the 10 other monosomic hybrid F_1 plants (Mo7xPima 3-79, Mo31xPima 3-79, Mo38xPima 3-79, Mo59xPima 3-79, Mo66xPima 3-79, Mo69xPima 3-79, Mo72xPima 3-79, Mo73xPima 3-79, Mo75xPima 3-79, and Mo79xPima 3-79), each deficient for one unknown chromosome showed the presence of only the polymorphic *G. barbadense*-specific SSR marker bands for BNL2572, CIR122, and GH-048, was lacking the respective missing L-458 alleles.

Because all of these SSR markers previously were assigned to chromosome 4 A_t -subgenome of cotton [11, 19–27], the results indicated that the unknown monosomes Mo7, Mo31, Mo38, Mo59, Mo66, Mo69, Mo70, Mo71, Mo72, Mo73, Mo75, Mo76, Mo79, Mo81, Mo89, and Mo90 all involved monosomy for chromosome 4 of the cotton A_t -subgenome (**Figure 2**).

Further, our results revealed that two monosomic hybrid F_1 plants (Mo13xPima 3-79 and Mo67xPima 3-79), each of which deficient for unknown chromosome, showed differential presence of polymorphic *G. barbadense*-specific SSR markers BNL1064, BNL2884, BNL3650, CIR203, Gh032, Gh039, Gh082, and TMB1538 and were missing the L-458 allele. Our results indicated that the monosomic chromosome in Mo13 and Mo67 is chromosome 6 A_t -subgenome of cotton because previous assignment of these differential SSR markers has been assigned to this chromosome [11, 19–27] (**Figure 3**).

Using the similar approach, monosomic line Mo48 was assigned to the chromosome 18 of the D_t -subgenome of cotton based on polymorphism of BNL3280 marker from chromosome 18. Monosomic line Mo17 was assigned to the chromosome 20 or 22 of the D_t -subgenome using chromosome-specific SSR marker—JESPR235 genotyping experiment with monosomic F_1 substitution hybrids (Mo17x Pima 3-79) [19, 26], because of this SSR marker was assigned to these two chromosomes.

A monotelodisomic line recovered among progeny of Mo21 and was identified using the chromosome-specific SSR markers—BNL3442, Gh246, CIR212 that were polymorphic monosomic F_1 substitution hybrids (Telo21x Pima 3-79). This result suggested that monotelodisomic line Telo21 is chromosome arm 11 of the A_t -subgenome of cotton because these SSR markers are from the arm of chromosome arm 11 of cotton [11, 19–27].

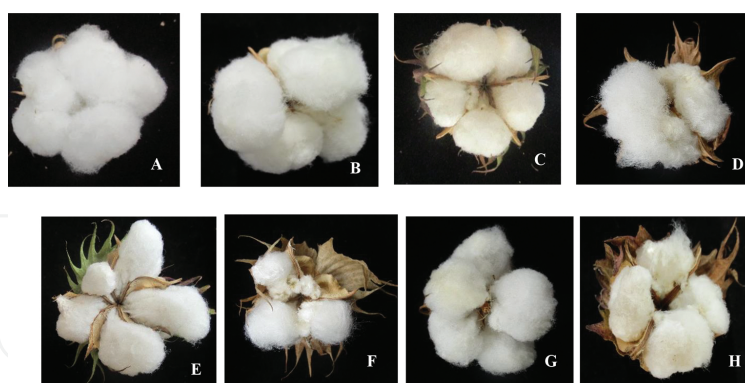


Figure 4. Examples of bolls from cotton monosomic lines with identified monosomes: (A) L-458-parental line; (B) Mo11; (C) Mo16; (D) Mo76; (E) Mo90; (F) Mo13; (G) Mo48; and (H) Mo17.

Thus, we identified chromosomes specificities for 24 monosomic and one monotelodisomic line using F_1 substitution hybrids and chromosome-specific SSR markers. Results demonstrated the differential rates of monosome occurrence and/or recovery among the cotton chromosome complement. In our Uzbekistan-based experiments, chromosome 2, 4, 6, 11, 18, and 20 or 22 were recovered as monosomic/monotelodisomic individuals. The identities of 11 additional F_1 substitution hybrids have yet to be identified, namely Mo1, Mo4, Mo9, Mo28,

Mo39, Mo50, Mo62, Mo82, Mo84, Mo91, and Mo92. Individual cotton chromosome deficiency had a specific influence in plant morphology and some characters such as bolls (**Figure 4**).

For instance, monosomic lines of the chromosome 2 A_t -subgenome including Mo11, Mo16, Mo19, and Mo93, all have a similar phenotypic syndrome that includes features such as dwarf plant architecture; small, narrow, and dense leaf; short sympodia and small and round bolls.

Monosomic lines of the chromosome 4 A_t -subgenome including Mo7, Mo31, Mo38, Mo59, Mo66, Mo69, Mo70, Mo71, Mo72, Mo73, Mo75, Mo76, Mo79, Mo81, Mo89, and Mo90, all have such characters as bushy plant; leaves usually with wavy margins; long peduncle and bolls; and long bolls. Monosomic lines of the chromosome 6 A_t -subgenome including Mo13 and Mo67—both have bushy plant; short sympodia; small and rounded bolls; and late flowering (**Figure 5**).

Monosomic plants deficient for a copy of chromosome 11 of the A_t -subgenome (Mo21) have dwarf plant architecture; short sympodia; small leaves; and small and defective bolls with curved apical tips. Monosomic plants deficient for a copy of chromosome 18 of the D_t -subgenome (Mo48) have dwarf plant architecture, short sympodia, small leaves and bolls that are smaller and rounder. Monosomic plants deficient for a copy of chromosome 20 or 22 of the D_t -subgenome (Mo17) have dwarf plant architecture; small leaves; short sympodia; small, defective, unsymmetrical bracteoles; late flowering and set fewer seed.

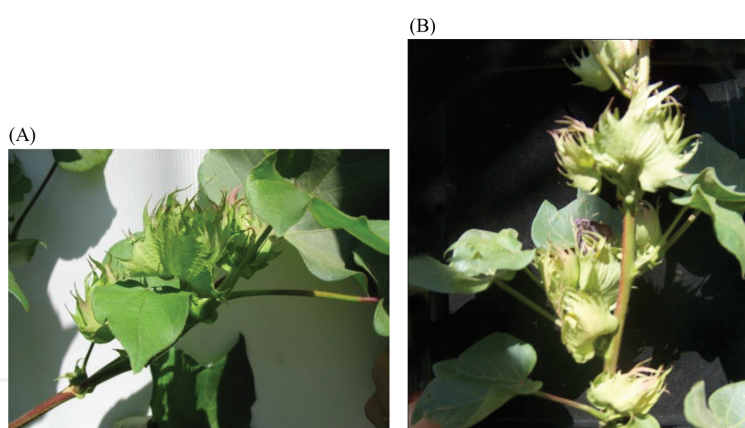


Figure 5. Some unique morphologic characters of the cotton monosomic lines with identified monosomes: (A) short sympodia on the monosomic line Mo19 (on chromosome 2) and (B) fragment of the stem of the monosomic line Mo67 (on chromosome 6).

3.3. Identification and numeration of the unknown monosomes

We have begun a new effort to identify cotton monosomic lines of the Uzbek cytogenetic cotton collection using a well-defined tester set of translocation lines of the cytogenetic collection of the USA, kindly provided by Prof. D.M. Stelly, Texas A&M University, USA, through USDA–Uzbekistan cotton germplasm exchange program. We performed numerous sexual crosses between monosomic lines of our collection and tester-translocation lines, but some monosomic lines were distinguished by low crossing characteristics and few seed set. Monosomes were

identified by analyzing meiotic metaphase I configurations of monosomic translocation heterozygous F_1 hybrids. When monosome of such a hybrid involves neither of the translocated chromosomes, each metaphase I cell typically includes a quadrivalent and a monosome ($23 \text{ II} + 1 \text{ IV} + 1 \text{ I}$). In contrast, when the monosome involves one of the translocated chromosomes, a trivalent typically occurs ($24 \text{ II} + 1 \text{ III}$) [29].

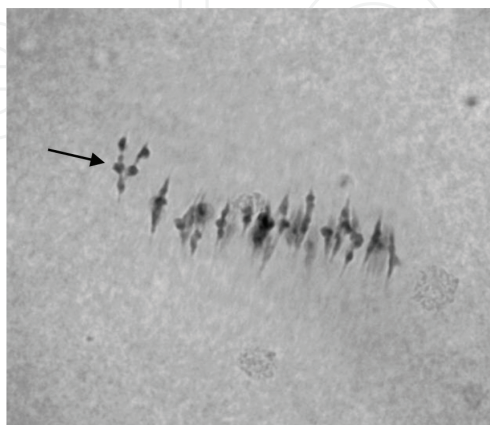


Figure 6. “Critical configuration” of the chromosomes at the meiotic metaphase I, showing 24 bivalents and 1 trivalent in cotton F_1 plant from the cross of monosomic Mo19xTT2L-6R (arrow point to the trivalent).

Cytological analyses of the two hybrid combinations involving two tester set lines (Mo19xTT6L-7L, Mo19xTT10R-11R) showed that monosome Mo19 is not chromosome 6, 7, 10, or 11 because the modal MI pairing configuration in the respective monosomic F_1 hybrids included $23 \text{ II} + 1 \text{ IV} + 1 \text{ I}$. In contrast, the tests with translocation lines TT2L-6R and TT2R-8Rb showed that the monosome Mo19 is chromosome 2, because the modal MI pairing configurations in the monosomic F_1 hybrids of Mo19xTT2L-6R and Mo19xTT2R-8Rb M I were $24 \text{ II} + 1 \text{ III}$ (**Figure 6**).

Since the monosomic line Mo19 had common chromosome 2 in the two translocation lines and it was assigned to chromosome 2 A_t -subgenome of cotton using SSR markers (BNL3971, BNL3590, and GH-198), we can now confidently tell that the monosome Mo19 from chromosome 2 A_t -subgenome (**Table 1**).

The cytological analyses of the two hybrid combinations involving two tester lines (Mo67xTT3R-5R, Mo67xTT9R-25) showed that monosome Mo67 is not chromosome 3, 5, 9, or 25 because the modal MI pairing configuration of its monosomic F_1 hybrids was $23 \text{ II} + 1 \text{ IV} + 1 \text{ I}$. The test with translocation line TT6L-7L showed, however, that the monosome Mo67 could be from chromosome 6 or 7, because in the monosomic F_1 hybrid of Mo67xTT6L-7L MI the modal MI pairing configuration was $24 \text{ II} + 1 \text{ III}$. Molecular marker data suggested that the monosome Mo67 must be chromosome 6 of the A_t -subgenome (**Table 1**).

The cytological test of the hybrid combination involving one tester line (Mo75xTT4R-15L) showed that monosome Mo75 could be from chromosome 4 or 15, because in the monosomic F_1 hybrid of Mo75xTT4R-15L MI pairing was $24 \text{ II} + 1 \text{ III}$. Molecular marker data suggested that the monosome Mo75 is chromosome 4 A_t -subgenome (**Table 1**; **Figure 7**).

Monosomic line	Origin	Year of the obtained	Chromosome		Molecular markers
			Size	Identity	
Mo11	Pollen irradiation	1991	Medium	A 2	BNL3590, GH-198
Mo16	Pollen irradiation	1991	Medium	A 2	BNL3971, BNL3590, GH198
Mo19	Pollen irradiation	1991	Large	A 2	BNL3971, BNL3590, GH198
Mo93	Pollen irradiation	2007	Medium	A 2	BNL1434, BNL1897, BNL3971
Mo7	Pollen irradiation	1990	Medium	A 4	BNL2572, CIR122, CIR048
Mo31	Pollen irradiation	1993	Medium	A 4	BNL2572, CIR122, CIR048
Mo38	Pollen irradiation	1993	Large	A 4	BNL2572, CIR122, CIR048
Mo59	Desynapsis	1996	Medium	A 4	BNL2572, CIR122, CIR048
Mo66	Pollen irradiation	1995	Medium	A 4	BNL2572, CIR122, CIR048
Mo69	Desynapsis	1997	Medium	A 4	BNL2572, CIR122, CIR048
Mo70	Desynapsis	1997	Medium	A 4	BNL2572, CIR122, GH-107
Mo71	Desynapsis	1997	Medium	A 4	BNL2572, CIR122, GH-107
Mo72	Desynapsis	1997	Medium	A 4	BNL2572, CIR122, CIR048
Mo73	Desynapsis	1997	Medium	A 4	BNL2572, CIR122, CIR048
Mo75	Pollen irradiation	1999	Medium	A 4	BNL2572, CIR122, CIR048
Mo76	Pollen irradiation	2001	Medium	A 4	BNL2572, CIR122, GH-107
Mo79	Desynapsis	2002	Small	A 4	BNL2572, CIR122, CIR048
Mo81	Pollen irradiation	2003	Medium	A 4	BNL2572, CIR122, GH-107
Mo89	Desynapsis	2003	Medium	A 4	BNL2572, CIR122, GH-107
Mo90	Pollen irradiation	2003	Small	A 4	BNL2572, CIR122, GH-107
Mo13	Pollen irradiation	1991	Large	A 6	BNL1064, BNL2884, BNL3650, CIR203, Gh032, Gh039, Gh082, TMB1538
Mo67	Translocation	1996	Large	A 6	BNL1064, BNL2884, BNL3650, CIR203, Gh032, Gh039, Gh082, TMB1538
Telo Mo21	Pollen irradiation	1991	Large	A 11	BNL3442, GH246, CIR212
Mo48	Pollen irradiation	1994	Small	D 18	BNL3280
Mo17	Pollen irradiation	1991	Medium	D 20 or 22	JESPR235

Table 1. Origin and some characters of the cotton monosomic lines of cotton *G. hirsutum* L.

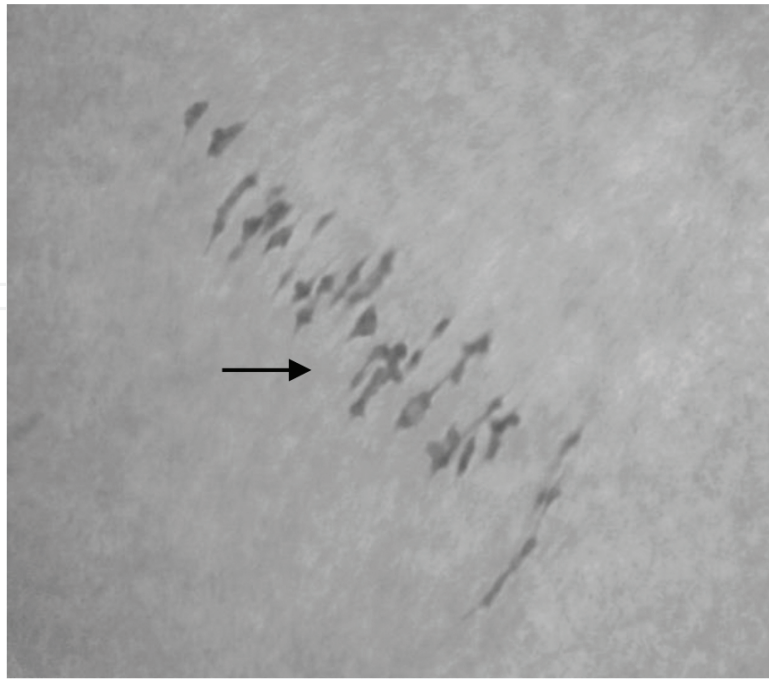


Figure 7. “Critical configuration” of the chromosomes at the meiotic metaphase I, showing 24 bivalents and 1 trivalent in cotton F_1 plant from the cross of monosomic Mo75 with TT4R-15L (arrow point to the trivalent).

Thus, out of one monotelodisomic and 24 monosomic lines identified using chromosome-specific SSR markers, three monosomes were confirmed by means of the translocation test. From 24 identified monosomic lines, four are chromosome A_t -2, 16 are chromosome A_t -4, two are chromosome A_t -6, and one each is chromosome D_t -18, chromosome D_t - 20 or 22 and telosome A_t -11.

4. Discussion

The utilization of the microsatellite markers and translocation lines for the identification of unknown cotton monosomic lines in our study provided the unified identification and numeration of the chromosomes and chromosome arm for 24 aneuploid lines of the Uzbek cytogenetic collection of cotton. On the basis of molecular-genetic and cytogenetic tests involving translocations, three of the six monosomes were designated chromosomes 2, 4, and 6 of the A_t -subgenome and two chromosomes were designated chromosomes 18 and 20 or 22 D_t -subgenome. One telosome was designated as a telosome 11 A_t -subgenome.

Twenty other monosomes were identified as duplicates of three of aforementioned monosomes (chromosomes 2, 4, and 6). Chromosome 4 of the A_t -subgenome was recovered more frequently (16 times), than chromosome 2 of the A_t -subgenome (4 times) and chromosome 6 of the A_t -subgenome (2 times) during the pollen irradiation in different doses and in progeny of the desynaptic plants (**Table 1**). The other chromosomes were recovered only once (chromosomes 18 and 20 or 22 D_t -subgenome and one telosome as a telosome 11 A_t -subgenome). These data

demonstrated differences between the studied chromosomes on the response to irradiation treatments, and/or differences in their ability to survive as embryos and plants, and/or their ability to be maternally transmitted through nullisomic maternal gametes and gametophytes. It is possible that the centromeric region of the cotton chromosome 4 is more susceptible to treatments, and/or this chromosome is under less severe selection against monosomy and thus deficiencies are recovered and transmitted more frequently in the natural and irradiated populations. Identification of other monosomic lines using similar strategy is in progress in our laboratories.

According to our previous results, two monosomics lines (Mo11 and Mo19) from our collection may be involved the same monosome and were homologous because they had chromosomal associations with the same translocation line Tr16 of Uzbek cytogenetic collection [12, 15].

There are many challenges in genetic and molecular-genetic analyses of the complex genomes of cotton [5, 30–32], and the development of novel aneuploid lines for tetraploid cotton will be important to solve mapping of molecular markers in the cotton genome. Our results, which are largely based on radiation-induced aneuploidy, paralleled the findings of Endrizzi et al. [16] showed that chromosomes 4 and 6 are the two chromosomes that occur most frequently as spontaneous monosomes in natural populations of cotton. A challenge in attaining complete genome coverage, that is, monosomes for all chromosomes may be that some chromosomes may contain genes that are unique and essential to the zygote, plant, functional megaspore or megagametophyte. For these chromosomes, it may not possible to recover or sexually utilize monosomes; for others, the difficulty is likely to range from relatively easy to challenging.

5. Conclusion

The results presented in this report suggest that microsatellite markers are facile and useful tools for cytogenetic analysis of the cotton chromosome deficient plants. The utilization of the microsatellite markers for the identification of unknown cotton monosomic lines provided an opportunity to foster the identification and numeration of the chromosomes and chromosome arm for 24 aneuploid lines of the Uzbekistan cytogenetic collection of cotton. These markers can also be readily used for the development of new cotton chromosome substitution lines and germplasm introgression.

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