

Chromosome pairing and genome size analysis in F₁ and F₂ offspring derived from crossing *Gossypium barbadense* and *G. hirsutum*

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Abstract

Cultivated cotton species *Gossypium hirsutum* L. and *Gossypium barbadense* L. are considered as the highest yield producing cottons, which are cultivated in several regions of Iran. Due to long time selection and cultivation practice, we need to broaden the cotton gene pool by producing new hybrids. Interspecific hybrids ensued after crossing *G. hirsutum* and *G. barbadense*. In the present study, cytogenetic characteristics and genome size of parental cotton species (*G. hirsutum* L. and *G. barbadense*) and their F₁ and F₂ hybrid progenies were studied. All cotton genotypes showed $2n = 4x = 52$ chromosome number. The genotypes differed significantly in cytogenetic characters like number of total intercalary, and terminal chiasmata, mean number of ring chromosomes and quadrivalents ($P < 0.05$). The genome size of the genotypes varied from 1.345 to 4.37 picograms. This

research revealed cytogenetic variability among the cotton offspring and if these are combined with agronomic characteristics, they can be used in cotton breeding.

Key words: Cytogenetic, *Gossypium*, hybridization.

Introduction

Cotton (*Gossypium hirsutum* L.) is the most important fiber crop plant throughout the world. Two cultivated allopolyploid cotton species *G. hirsutum* L. and *G. barbadense* L. produce about 98% the world cotton. Different cotton cultivars are grown in Iran and according to FAOSTAT (2011), cotton production has been recently increased to 140,000 t in this country.

Due to artificial selection and continuous cultivation of the same cultivars over a long period, genetic diversity decreased, which may lead to genetic erosion. Useful alleles may be lost because of this genetic erosion, which in turn leads to plant vulnerability to pests and pathogens (Vafaie-Tabaret et al., 2003; Mehetre et al., 2003; Ranaand Bhat, 2005; Dongre et al., 2007; Sheidai et al., 2008; Wei et al., 2008; Sheidai et al. 2010; Noormohammadi et al., 2013).

Interspecific and intraspecific hybridization are considered as an immediate strategy to boost genetic diversity in cotton. *G. barbadense* with suitable fiber quality plays a major role in cotton hybridization (Wang et al., 2011).

Recently interspecific hybrids between *G. hirsutum* and *G. barbadense* were made in the Gorgan Cotton Research Center at Iran. We began studying genetic variability of these new genotypes by using molecular and cytogenetic analyses. Cytogenetic research in cotton are mainly concerned with chiasma frequency and distribution as well as chromosome pairing (Sheidai, 2008; Sheidai and Dezfolian, 2008; Sheidai et al., 2006;

Tafvizi et al., 2010; Noormohammadi et al., 2013). Genome size is also a useful characteristic for species delimitation or hybrid identification (Keller et al., 1996; Buitendijk et al., 1997; Bare et al., 1998). In this study, we considered cytogenetic characteristics and genome size (C-value) variation in cotton genotypes of *G. hirsutum*. and *G. barbadense* and their derived F₁ and F₂ offspring.

Materials and Methods

Sampling and cytogenetic study

Nine genotypes of cotton tetraploid species (*G. hirsutum* L. and *G. barbadense* L.) were used in present study. These genotypes were the parental genotypes Siokra and Sahel (*G. hirsutum* L.); Barbadense 5595 and Termez14 (*G. barbadense* L.); F₁ hybrids and F₂ offspring from Termez-14 × Siokra, Barbadense 5595 × Sahel, Termez-14 × Sahel and Barbadense 5595 × Siokra (Table 1).

These cotton plants were cultivated in three rows of 10 m length with 20 cm interplant distance, in the experimental field of Gorgan Cotton Research Center, following a completely randomized design (CRD) with 3 replications. Fifty flower buds were randomly collected from 10 randomly selected plants in each accession or cultivar. The squash technique was used for cytogenetic analyses. The chromosomes behavior and chiasma frequency were studied in different stages of meiosis. Pollen stainability as a measure of pollen fertility was according to Sheidai et al. (2008).

Genome size analysis

The genome size was determined by flow cytometry. There were three readings per each plant. The nuclei suspensions were prepared from small amount of mature fresh leaf tissue together with an equal weight of mature leaf tissue of the external standard. The external standard used for tetraploid cotton was *Allium cepa* cultivar Ailsa Craig (2C = 33.55 pg).

The leaves were chopped with a single-used sharp scalpel with adding 400 μ l nuclei isolation buffer in the plastic petri dish at room temperature. To staining the nuclei, 1600 μ l DNA fluorochrome or DAPI (2, 6-Diamidino-2-phenylindole) was added and suspensions was filtered through a 50 μ m nylon mesh into a labeled sample tube. Stained nuclei suspensions were analyzed with a Partec Flow Cytometer (Partec Germany). The flow cytometric statistics such as: coefficient of variation (CV), mode, mean and the number of cells counted in each sample, are included in the histograms obtained.

DNA amounts are measured in picograms (pg) and the status of nuclei is described in terms of 'C' values (Doležel et al., 2007). 1 pg of DNA represents 978 mega base pairs (Mbp). The amount of nuclear DNA of each sample was calculated based on the values of the G1 peak means (Doležel and Bartoš, 2005).

Data analysis

The analysis of variance (ANOVA) was performed to show difference in 2C-value content among the plants. The Unweighted Paired Group with Average Method (UPGMA) clustering used standardized cytological data (mean = 0, variance = 1). Principal components analysis (PCA) was performed to show the genotype distinctness in meiotic features (Podani, 2000; Sheidai, 2008). Pearson coefficient of correlation was determined among cytological characters. SPSS ver. 18 (2009) and PASTver. 2.17c (2013) softwares were used for statistical analyses.

Results

Cytology

Cytogenetic research on all samples was successfully carried out except with Termez14 parental genotype. Hence, we removed it from our further study. All cotton genotypes

showed $2n = 4x = 52$ chromosome number (Figure 1). Diffuse stage (complete or partial) was observed in all cotton genotypes. All genotypes formed mostly bivalents, although some amount of univalents and quadrivalents were also formed (Table 1). The highest value of total chiasmata (33.36), terminal chiasmata (33.31) and ring bivalent (9.05) occurred in Termez-14 × Siokra F₁ plants while the highest value of intercalary chiasmata (0.4) and number of quadrivalents (0.93) occurred in Barbadense5559 and Siokra respectively (Table 1).

Table 1. Meiotic characteristics of the cotton cultivars.

| No | genotype | TOX | IX | TX | UNI | ROD | RB | IV | PF% |
|----|-------------------------------|-------|------|-------|------|-------|------|------|-------|
| 1 | Barbadens 5559 | 28.50 | 0.40 | 28.01 | 2.59 | 18.21 | 4.84 | 0.18 | 96.90 |
| 2 | Siokra | 27.86 | 0.20 | 27.66 | 3.50 | 16.53 | 4.00 | 0.93 | 98.90 |
| 3 | Sahel | 29.00 | 0.13 | 28.86 | 3.10 | 16.60 | 4.70 | 0.73 | 98.20 |
| 4 | Termez-14 x Siokra (F1) | 33.36 | 0.05 | 33.31 | 2.15 | 14.36 | 9.05 | 0.21 | 98.80 |
| 5 | Barbadense 5559 x Sahel (F1) | 30.56 | 0.03 | 30.53 | 1.73 | 17.93 | 5.86 | 0.23 | 99.70 |
| 6 | Termez-14 x Sahel (F1) | 30.00 | 0.00 | 30.00 | 2.12 | 17.61 | 7.61 | 0.32 | 98.50 |
| 7 | Termez-14 x Siokra (F2) | 26.37 | 0.12 | 26.25 | 4.25 | 17.12 | 4.31 | 0.00 | 98.10 |
| 8 | Barbadense 5559 x Sahel (F2) | 30.07 | 0.15 | 29.92 | 2.84 | 16.07 | 6.92 | 0.07 | 98.90 |
| 9 | Barbadense 5559 x Siokra (F2) | 30.86 | 0.09 | 30.77 | 1.36 | 18.13 | 6.13 | 0.13 | 99.20 |

Abbreviations: TOX=Total chiasmata, IX=intercalary chiasmata, TX=terminal chiasmata, UNI= univalents, ROD=rod bivalents, RB=ring bivalents, IV=quadrivalent, PF=pollen fertility.

Meiotic abnormalities, including chromosome stickiness, laggard chromosomes, micronuclei formation, unorganized chromosome and multipolar cell formation, were observed in different stages of meiosis I and II in some cultivars. Barbadense5559 was the only genotype that showed the occurrence of all types of observed abnormalities. The other genotypes showed multipolar formation (Fig.1).

There were significant differences among the studied genotypes for meiotic characters such as total chiasmata, intercalary chiasmata, terminal chiasmata, and number of ring bivalents and quadrivalents ($P < 0.05$, Table 2). The ANOVA showed significant

difference for intercalary chiasmata ($P = 0.01$), among parental genotypes, F_1 plants and F_2 offspring

Pearson correlation showed significant positive correlation between ring bivalent and and terminal chiasmata ($r > 0.80$, $P < 0.01$). The mean value of univalent was negatively correlated with the mean total and mean terminal chiasmata per bivalent ($r > -0.70$, $P < 0.05$).

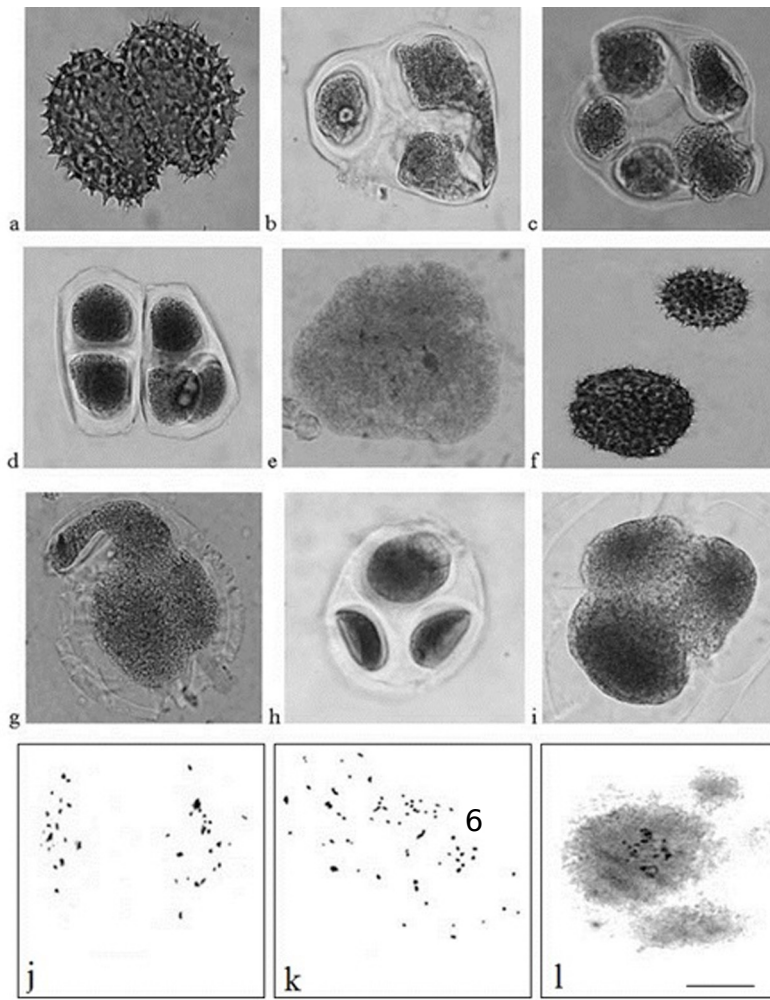


Fig. 1. Representative meiotic cells in cotton genotypes. a= abnormal pollen grain (Barbadense × Sahel ,F₂), b= tripolar cell (Barbadense × Sahel F₂), c= pantapolar cell (Barbadense × Sahel, F₂), d= octapolar cell (Barbadense × Sahel F₂), e= diffiusion stage (Barbadense 5559), f= 2n pollen grain (Barbadense x Sahel F₂), g= abnormal pollen grain (termez-14 × Siokra, F₂), h= tetrad (Siokra), i= tripolar (Barbadense × Siokra, F₂), j= anaphase (Barbadense×Siokra, F₁) , k=metaphase with univalent chromosomes (Barbadense×Sahel, F), and l= stickiness phenomena (Barbadense×Sahel, F₁). Scale bar_10mm

PCA plot (Fig.3) supported UPGMA tree result and separated parental plants, F₁ and F₂ plants from each other. This result along with ANOVA result showed cytogenetic distinctness of the studied cotton plant groups. Intercalary chiasmata, number of univalents and rod bivalents, separated parental cotton plants of *G. hirsutum* and *G. barbadense* from each other, while number of quadrivalent separated Sahel and Siokra cultivars (*G. hirsutum*) from each other.

Genome size

The genome size obtained in the cotton genotypes varied from 1.345 to 4.37 picograms (Fig. 4). The highest and lowest genome sizes were observed in Termez14 × Sahel F₂ plants and Barbadense5559, respectively. The mean genome size value obtained in parental plants was 2.23 pg while, it was 2.46 pg in F₁ and F₂ plants.

ANOVA test produced no significant difference ($P < 0.05$) in genome size among the studied cotton genotypes. We did not obtain significant correlation between genome size and cytogenetic characters.

| | | Sum of Squares | Degree of freedom | Mean Square | F | Sig. |
|-----|----------------|-------------------|----------------------|-------------|--------|------|
| TOX | Between Groups | 186.775 | 7 | 26.682 | 2.761 | .023 |
| | Within Groups | 309.200 | 32 | 9.663 | | |
| | Total | 495.975 | 39 | | | |
| IX | Between Groups | 7.900 | 7 | 1.129 | 10.032 | .001 |
| | Within Groups | 3.600 | 32 | .113 | | |
| | Total | 11.500 | 39 | | | |
| TX | Between Groups | 204.375 | 7 | 29.196 | 2.998 | .015 |
| | Within Groups | 311.600 | 32 | 9.738 | | |
| | Total | 515.975 | 39 | | | |
| Uni | Between Groups | 22.700 | 7 | 3.243 | 2.199 | .061 |
| | Within Groups | 47.200 | 32 | 1.475 | | |
| | Total | 69.900 | 39 | | | |
| ROD | Between Groups | 74.300 | 7 | 10.614 | 1.044 | .421 |
| | Within Groups | 325.200 | 32 | 10.163 | | |
| | Total | 399.500 | 39 | | | |
| RB | Between Groups | 96.175 | 7 | 13.739 | 3.701 | .005 |
| | Within Groups | 118.800 | 32 | 3.713 | | |
| | Total | 214.975 | 39 | | | |
| IV | Between Groups | 9.575 | 7 | 1.368 | 2.669 | .027 |
| | Within Groups | 16.400 | 32 | .513 | | |
| | Total | 25.975 | 39 | | | |

Fig. 2. UPGMA tree based on meiotic data. Numbers are according to Table 1

Table 2. ANOVA test based on cytogenetic parameters among cotton genotypes.

Abbreviations: TOX=Total chiasmata, IX=intercalary chiasmata, TX=terminal chiasmata, UNI= univalents ROD=rod bivalents, RB=ring bivalents, IV=quadrivalent, PF=pollen fertility

Discussion

Significant difference in chiasma frequency and chromosome pairing observed among the cotton genotypes partly indicate their genetic difference- it is considered as an important criterion in cotton genetic diversity (Sheidai et al., 2008; Noormohammadi et al., 2012). Chiasma frequency is genetically controlled and significant change in this cytogenetic feature leads to change in recombination and genetic variation within plant populations (Sheidai 2008). In this study, the highest value of terminal and total chiasma were observed in interspecies hybrid plants possibly due to the occurrence of higher amount of recombination within their genomes (A_t and D_t genomes).

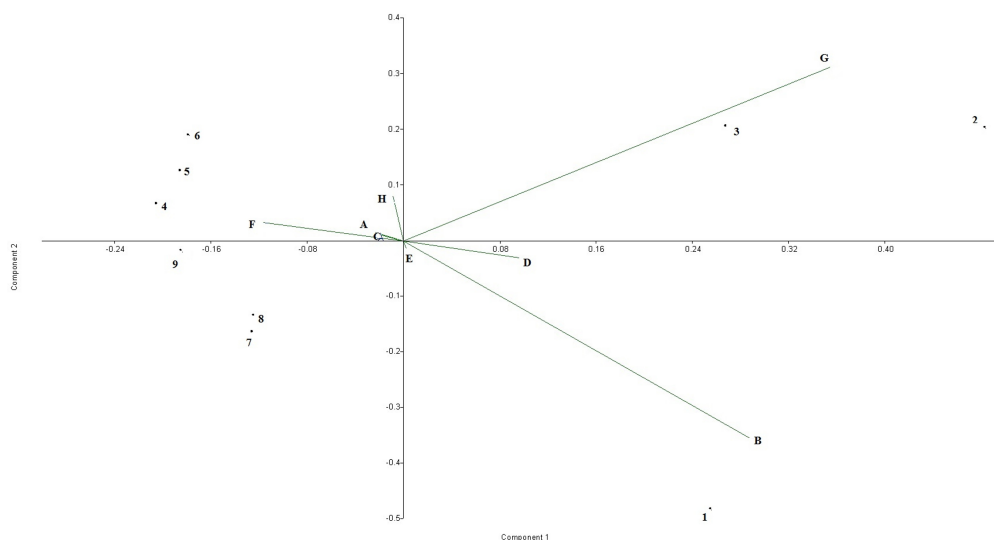


Fig. 3. PCA ordination based on cytogenetic data. Number are according to Table 1.

Letters: A= Total chiasmata, B= intercalary chiasmata, C=terminal chiasmata, D= univalent, E=rod bivalents, F=ring bivalents, G=quadrivalent and H=C value

Quadrivalents were formed in both studied parental and hybrid plants. Alternate and adjacent quadrivalents are known to occur in cotton cultivars (Sheidai, 2008). Usually cotton cultivars having type I adjacent quadrivalents show high pollen fertility due to the proper chromosomes segregation. In the present study, investigated genotypes had high pollen fertility (> 95 %). Therefore, they had type I adjacent quadrivalents.

Sheidai (2008) and Noormohammadi et al. (2013) also reported type I and II adjacent quadrivalents in tetraploid cotton cultivars (*G. hirsutum*) and their hybrid progenies.

Different chromosome abnormalities were observed in Barbadense5559 cultivars while other genotypes showed only multipolar cell formation. Such meiotic abnormalities may lead to the formation of abnormal tetrads and aneuploid gametes (Sheidai, 2008; Noormohammadi et al., 2012).

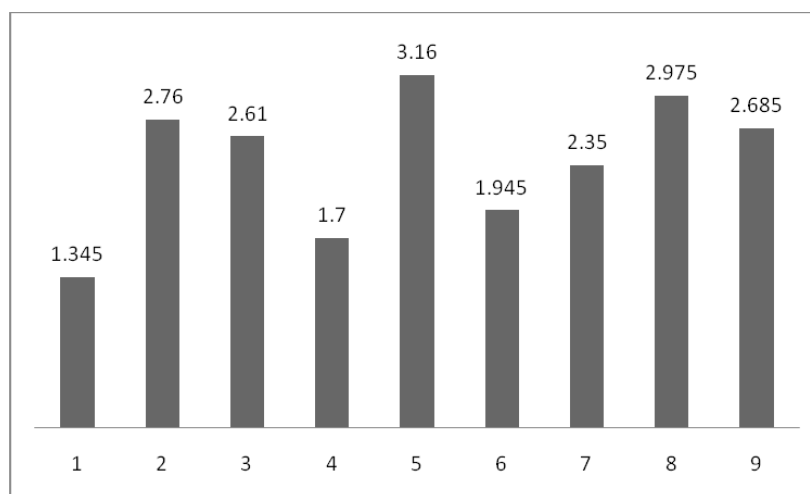


Fig. 4. Genome size (pg) in cotton genotypes. Numbers are according to Table 1

In UPGMA tree, F1 and F2 plants showed similarity to Siokra and Sahel, (*G. hirsutum*) parental plants. However, parental plants of Barbadense5559 (*G. barbadense* L.) stood far from its hybrid progenies. It may indicate different interactions of two cotton species genomes in the produced hybrid plants.

F2 interspecific hybrid plants showed higher 2C-values compared to the parental and F1 plants. The occurrence of genetic recombination and transposable elements expansion is known as causes of variation among cotton plants' genomes size (Hawkins et al., 2009). Intraspecific C-value variations have been also reported in other species (Murray, 2005). Therefore, from cytogenetic points of view, both significant change in the genes controlling chiasma frequency and formation as well as change in the genome size, indicate genetic difference of the studied cotton genotypes. Such data may be used in further selection and hybridization program in cotton.

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