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Cytogenetic distinctiveness of sixty-six tetraploid cotton (*Gossypium hirsutum* L.) cultivars based on meiotic data

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A cytogenetic study was performed on 66 tetraploid cotton cultivars (*Gossypium hirsutum* L.) and their hybrids. The cultivars studied differed significantly in their chiasma frequency and distribution as well as chromosome pairing indicating their genetic differences. Adjacent and alternate quadrivalents were formed in most of the cultivars. A-A or D-D heterozygote translocations occurred in most of the cultivars, while A-D (V-type) heterozygote translocations occurred in the cultivars Sahel, Oltan X Sahel and Modified. The cultivars B557, Modified, Sahel and Tashkand showed the occurrence of chromosome migration and aneuploid meiocytes. B-chromosomes occurred in some of the cultivars. Clustering of the cultivars showed distinctness of some of the parental genotypes and their hybrids. The Cytogenetic differences observed if combined with morpho--agronomic characters may be used in cotton breeding.

Keywords: cotton, cytogenetic, cytomixis.

Introduction

Tetraploid cotton (*Gossypium hirsutum*) with the genome constitution 2 $(AD)_1$ (2n = 52) along with *G. barbadense* dominate the world cotton production. The genomes of *G. hirsutum* individually are referred to as A_h and D_h and their chromosomes as H1–H13 and H14–H26, respectively (MENZEL and BROWN 1978).

Cotton is considered as one of the most important crop plants in Iran, cultivated in various regions of the country. Continuous cultivation of the same genotypes may bring about genetic erosion in the long term; therefore study of the available genetic variability as well as introducing the new ones is of importance. The present report considers the cytogenetic study of 66 cotton cultivars cultivated in Iran, dealing with their meiotic peculiarities including chiasma frequency and distribution, the types of quadrivalents (heterozygote translocations), B-chromosomes and the possible occurrence of unreduced gametes.

Materials and methods

Sixty six tetraploid cotton cultivars (*Gossypium hirsutum*) were cultivated in three rows of 10 m length with 20 cm interplant distance, in the experimental field of the Varamin Cot-

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ton Research Center of Iran, according to a completely randomized design (CRD) with 3 replications. For cytogenetical studies, fifty flower buds were used randomly from 10 randomly selected plants of each line making the total collection $50 \times 10 \times 10 = 5000$. The squash technique and pollen fertility test used followed the earlier report (SHEIDAI et al. 2005).

In order to determine a significant difference in cytogenetic characteristics as well as morphological characteristics the analysis of variance (ANOVA) followed by the least significant difference test (LSD) as well as χ^2 test were performed on the genotypes. The grouping of the cultivars was performed by using different clustering methods as well as ordination based on principal coordinate analysis (PCO). For cluster analysis, standardized data (mean = 0, variance = 1) and Taxonomic distances were used. In order to identify the most variable meiotic characteristics among the genotypes studied, factor analysis based on principal components analysis (PCA) was used (SHEIDAI and ATTAEI 2005). Statistical analysis used SPSS ver.9 (1998) and NTSYS ver. 2.02 (1998) softwares.

Results

Diffuse stage in meiosis-I prophase

The meiotic analysis of all cotton cultivars studied showed the occurrence of a post pachytene diffuse stage (Fig. 1, A). Despiralization of chromosomes occurred after pachytene, commencing the diffuse stage. The diffuse stage may be of the complete type in which whole chromosomes decondense or it may be partial, in which some parts of the genome show decondensation. The present study shows the occurrence of a partial/complete diffuse stage in the cotton cultivars.

Chiasma frequency and chromosome pairing

The highest value of total chiasmata occurred in the hybrid cultivar 1097 X 1106 (59.90) while the lowest value occurred in the hybrid cultivar Oltan X Tashkand (28.00). The highest value of terminal chiasmata (Tab. 1) occurred in the cultivar S-150 (43.00) while the lowest value occurred in C-200 (29.00). The highest value of intercalary chiasmata occurred in the cultivar C15-4 (16.39) while the cultivars Sayer 314, Color fiber, Siokra, 1097, 1106 as well of some of their hybrids showed no intercalary chiasma formation. ANOVA and LSD tests performed on chromosome pairing and chiasma frequency revealed a significant difference among the cultivars studied for almost all meiotic characters.

The cotton cultivars studied formed mainly ring and rod bivalents in the metaphase of meiosis-I (Fig. 1, B, C, G, H), while univalents were formed in some of the cultivars (Fig. 1, H), trivalent formation was confined to the cultivars No-228, Color fiber X Bakhtegan and Color fiber X Tashkand (Tab. 1).

Quadrivalents were formed in most of the cultivars studied (Tab. 1, Fig. 1, B). Adjacent quadrivalents were formed in Bakhtegan X 1106, Bakhtegan X 1097, Tashkand X Siokra, B557 X Color fiber, Siokra X 1097, as well as Chirpan cultivar and its hybrids. Almost all these cultivars possessed high pollen fertility (98.70–99.50%).

Tab. 1. Cytogenetic characteristics of the cotton cultivars.

Cultivar	TOX	IX	TX	RB	RD	I	IV	III	VI
1- Gukurova	28.67	28.13	0.53	5.87	14.87	7.87	0.67	0.00	0.00
2- Nazili84	35.24	35.20	0.04	8.68	16.36	0.32	0.40	0.00	0.00
3- No 228	31.78	30.94	0.83	3.56	19.39	0.00	1.28	0.11	0.06
4- No 200	45.00	44.93	0.07	19.36	6.07	1.00	0.00	0.00	0.00
5- Sayer314	33.78	33.78	0.00	7.35	18.22	0.00	0.22	0.00	0.00
6- Tabladilla	34.00	33.76	0.24	7.04	17.52	0.08	0.60	0.00	0.04
7- Varam77	32.38	30.75	1.63	3.19	18.36	0.13	2.00	0.13	0.00
8- VXSX4S4	28.57	28.33	0.24	2.05	23.05	0.10	0.43	0.00	0.00
9- VXSXO	30.40	29.83	0.57	3.20	20.77	0.20	0.97	0.00	0.00
10- Sahel77	31.08	29.92	1.17	2.92	19.75	0.08	1.58	0.08	0.00
11- C15-1	30.83	11.83	19.00	64.10	35.90	0.10	0.34	0.00	0.00
12- C15-2	31.30	13.50	17.80	60.38	39.62	0.20	0.33	1.69	0.00
13- C15-3	34.00	15.00	19.00	65.38	34.62	0.00	0.36	0.00	0.00
14- C15-4	36.22	16.39	19.83	67.61	32.39	0.00	0.28	1.54	0.00
15- C15-5	33.07	12.13	20.93	70.59	29.41	0.00	0.14	1.00	0.00
16- C15-8	37.32	16.06	21.25	74.76	25.24	0.00	0.19	2.00	0.00
17- C35-9	31.23	15.39	15.85	57.05	42.95	0.00	0.18	0.00	0.00
18- C35-10	31.44	14.06	17.39	65.99	34.01	0.00	0.08	2.38	0.00
19- C35-11	28.15	10.25	17.90	58.08	41.92	0.30	0.08	2.70	0.00
20- Chirpan	35.18	13.55	21.64	76.57	23.43	0.00	0.05	0.00	0.00
21- Var76	46.33	4.03	45.40	21.30	3.20	0.40	1.00	0.00	0.00
22- T300	40.60	3.45	37.65	16.80	0.65	0.45	1.20	0.00	0.35
23- S150	44.20	1.25	43.95	21.65	2.40	0.00	0.15	0.00	5.00
24- C250	42.25	2.95	39.85	17.95	1.85	0.25	0.95	0.00	5.00
25- C200	31.55	1.85	29.80	14.35	1.75	0.00	5.00	0.00	0.15
26- OXZ	41.00	2.33	38.66	16.33	8.00	3.20	0.00	0.00	0.00
27- OXT	28.00	1.55	26.44	10.88	5.40	19.32	0.00	0.00	0.00
28- OXM	36.70	2.30	34.40	13.20	9.20	7.20	0.00	0.00	0.00
29- OXS	29.91	2.33	27.58	10.33	8.30	13.32	0.33	0.00	0.00
30- Z	42.89	3.73	39.21	17.00	6.42	5.14	0.00	0.00	0.00
31- T	41.50	3.21	38.28	16.07	7.28	5.28	0.00	0.00	0.00
32- M	35.58	4.00	31.58	13.30	8.41	8.50	0.00	0.00	0.00
33- S	35.18	2.90	32.27	13.63	5.45	13.44	0.09	0.00	0.00
34- O	42.57	2.78	39.78	16.78	7.05	3.46	0.21	0.00	0.00
35- OXB	33.66	2.16	31.50	12.25	8.50	10.16	0.08	0.00	0.00
36- BXS	40.20	7.07	33.13	15.13	6.13	1.73	0.00	0.00	0.40
37- BXM	36.80	7.10	29.70	16.00	5.00	2.10	0.00	0.00	0.30
38- BXT	37.89	8.33	29.56	15.78	6.56	1.33	0.00	0.00	0.33
39- BXZ	39.69	6.85	32.85	16.92	5.31	1.31	0.00	0.00	0.38
40- BXB557	40.71	8.00	33.57	16.71	5.00	1.43	0.00	0.00	0.43
41- B557	44.00	5.80	38.20	20.60	3.80	0.80	0.00	0.00	0.00

Tab 1. – continued

Cultivar	TOX	IX	TX	RB	RD	I	IV	III	VI
42- B	41.71	7.00	34.71	19.00	4.57	1.00	0.00	0.00	0.14
43- T150	42.91	0.91	42.00	14.58	6.75	0.08	8.00	0.00	0.00
44- T200	47.25	0.63	46.63	18.50	3.62	0.00	1.50	0.00	0.12
45- T250	46.64	0.10	46.54	18.00	4.50	0.00	1.60	0.00	0.00
46- B200	42.66	0.66	42.00	15.33	6.66	0.66	1.30	0.00	0.00
47- Sahel76	38.24	2.32	35.92	15.40	9.52	0.08	2.00	0.00	0.00
48- Color fiber	51.40	0.00	51.40	24.20	1.00	0.00	0.80	0.00	0.00
49- Siokra	52.00	0.00	52.00	24.00	1.00	2.00	0.00	0.00	0.00
50-1097	52.00	0.00	52.00	25.40	0.00	0.00	0.30	0.00	0.00
51- 1106	52.00	0.00	52.00	26.00	0.00	0.00	0.00	0.00	0.00
52- CFXB	49.00	0.00	49.00	23.00	1.00	1.00	1.00	1.00	0.00
53- 1106XB	51.80	0.00	51.80	25.90	0.10	0.00	1.70	0.00	0.00
54- 1097XB	52.00	0.50	51.50	24.00	0.50	0.00	0.75	0.00	0.00
52- TXB557	49.00	0.60	48.40	25.50	0.00	0.00	0.25	0.00	0.00
53- CFXT	48.60	0.00	48.60	23.70	0.00	0.66	0.00	1.33	0.00
54- SXT	49.00	1.00	48.00	24.70	0.00	0.00	0.60	0.00	0.00
55- CFXB557	50.40	0.40	50.00	25.42	0.00	0.00	0.28	0.00	0.00
56- SXB557	50.00	0.60	49.40	24.20	0.10	0.00	0.20	0.00	0.00
57- 1106XB557	52.00	0.00	52.00	26.00	0.00	0.00	0.00	0.00	0.00
58- 1097XB557	48.44	0.44	48.00	24.00	1.20	0.60	0.15	0.00	0.00
59- CFXS	52.00	0.00	52.00	24.10	0.00	0.00	0.90	0.00	0.00
60- CFX1097	42.40	0.00	42.40	19.60	0.00	4.80	2.00	0.00	0.00
61- 1106XS	52.00	0.00	52.00	25.00	1.00	0.00	0.50	0.00	0.00
62- 1097XS	49.80	0.80	49.00	23.80	2.20	0.00	2.00	0.00	0.00
63- 1097X1106	59.90	0.90	59.00	18.70	1.30	0.00	1.50	0.80	0.00

TOX – Total chiasmata, IX – Intercalary chiasmata, TX – Terminal chiasmata, RB – Ring bivalents, RD – Rod bivalents, I – Univalent, IV – Quadrivalent, III – Trivalent, VI – Hexavalent.
Cultivars abbreviations: B – Bakhtegan, C – Chirpan, CF – Color fiber, M – Modified, O – Oltan, S – Sahel, T – Tashkand, Z – Zeta 2.

A-A or D-D heterozygote translocations occurred in some of the cotton cultivars (Tab. 1), while the cultivars Sahel, Oltan X Sahel and Modified possessed A-D (V-type) heterozygote translocations (Fig. 1, B). The cultivars Tabladilla, T300, S150, C200, C250, T200, Bakhtegan and its crossing progenies formed hexavalent in metaphase of meiosis-I (Tab. 1).

Meiotic abnormalities

Metaphase and anaphase chromosome stickiness occurred in the cultivars Zeta2, Sahel, Oltan X Sahel, B557, C-200, T-300 and Var-76, Shirpan, Bakhtegan and its crossing progenies as well as Chirpan and its crossing progenies (Fig. 1, K, L). The degree of chromosome stickiness ranged from stickiness among two or more chromosomes to the involvement of

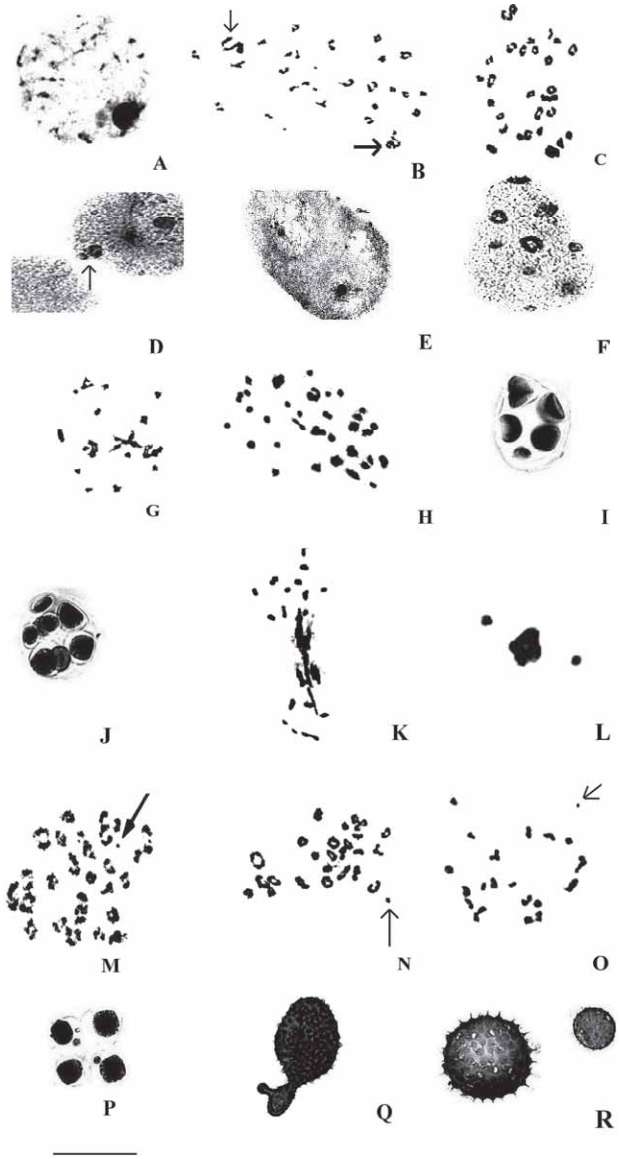

Fig. 1. Post pachytene diffuse stage in the cultivar Varamin 77 (A); Heterozygote V-type translocation (arrows) in the cultivar Sahel (B); Bivalent formation in the cultivar Sayer314 (C);. Cytomixis between two neighboring meiocytes in the cultivar B557 (D); Meiocyte in the cultivar Modified showing two sets of genomes due to cytomixis (E); Multipolar cell in the cultivar Tabladilla (F); Aneuploid meiocyte showing reduction in chromosome number in the hybrid cultivar Bakhtegan X B-557 (G); Univalent formation in the cultivar Gukurova (H); Abnormal tetrads in the cultivars T-300 and Gukurova (I, J); Chromosome stickiness in the cultivars Bakhtegan X Sahel and Zeta2 (K. L); B-chromosome (arrow) in the cultivars Tabladilla, Gukurova and Oltan X Tashkand (M-O); Micronuclei in tetrad cells of the cultivar Tashkand (P); Abnormal pollen grain in the cultivar Bakhtegan (Q); Potential unreduced pollen grain (large size pollen) in the cultivar C-200 (R). Scale bar indicates 10 μm

all metaphase chromosomes forming a complete clump (Fig. 1, L). Some of the cotton cultivars showed the occurrence of 1 to a few laggard chromosomes in anaphase I and II as well as telophase-I and II.

In some cases the hybrids showed lower chromosome pairing and pollen fertility compared to the parental genotypes, for example the parental genotypes of B557, 1097 and Color fiber formed bivalents in metaphase of meiosis-I, while their hybrids i.e. B557 X1097 and 1097 X Color fiber formed univalents in the metaphase of meiosis-I and possessed a lower pollen fertility. The univalents formed may be responsible for micronuclei formation observed in tetrads (Fig. 1, P). However some hybrids such as Color fiber X Siokra and Siokra X B557showed improvement over both parental genotypes with regard to the number of univalents and percentage of pollen fertility.

Multipolar cells, abnormal tetrads and pollen grains were observed in some the cultivars (Fig. 1, I, Q). Pair-wise χ^2 test performed showed a significant difference in the percentage of cells showing meiotic abnormalities among the cultivars studied.

Bakhregan cultivars and its crossing progenies, as well as B557, Modified, Sahel, Tashkand, Zeta 2 and hybrids of Chirpan cultivar showed the occurrence of chromosome/chromatin migration in different directions from early prophase to late telophase II (Fig. 1, D, E). These cultivars also showed aneuploid meiocytes possessing extra as well as reduced number of chromosomes in metaphase of meiosis-I and also formation of deformed and infertile pollen grains (Fig. 1, G, H).

In the cultivars C-250, C-200 and T-300 some of the pollen grains formed were bigger in size, ranging from 50–70 μm in diameter (Fig. 1, R), and differed significantly ($p < 0.01$) from the rest of the pollen grains (40–50 μm in diameter). Similarly in the cultivar Gukurova about 2% of the pollen grains formed were significantly larger in size, (110–130 μm in diameter) than the other pollen grains (80–95 μm in diameter). The presence of giant pollen grains has been used as an indication of the production of 2n pollen

B-chromosomes

The cotton cultivars Sahel, Sahel 77, Tashkand, Oltan X Tashkand, Oltan X Zeta2 and Oltan X Sahel, Tabladilla, Gukurova, Sahel 77 X Okra, Sahel 77 X 4-S-4 and No 228 showed the presence of 0-4 B-chromosomes (Bs) (Fig. 1, N, O). The B-chromosomes were smaller than the A-chromosomes and did not form any meiotic association with them. B-chromosomes could arrange themselves, along with the A-chromosomes, on the equatorial plane of the spindle and move to the poles during anaphase to be included in the next generation gametes. The presence of B-chromosomes in the hybrid genotypes studied supports transmission of the Bs from parents to the next generation.

Grouping of the cultivars

Different methods of cluster analysis as well as ordination of the cotton cultivars produced a similar grouping (Figs. 2, 3). Some of the parental genotypes and their hybrids formed distinct clusters indicating genetic differences from the other genotypes. For example, Chirpan cultivar and its crossing progenies (except C250) show similarity and are placed in a single cluster (Fig. 2); the same is true for most of the B557 hybrids.

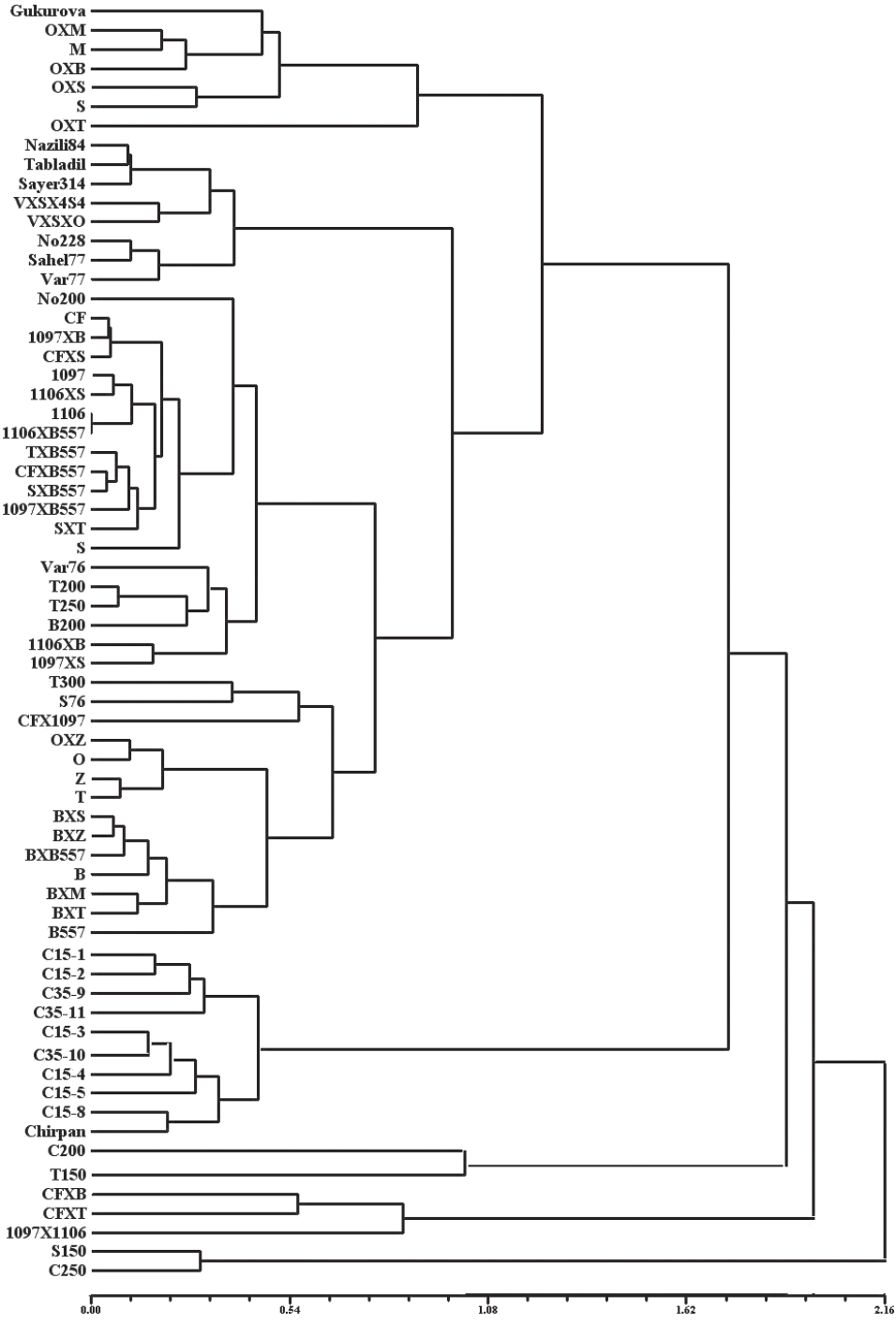

Fig. 2. UPGMA clustering of the cotton cultivars based on cytogenetical data (the cultivars name as in Tab. 1).

The cultivar Oltan showed similarity to its hybrid with Zeta 2 (O X Z) while, its other crossing progenies are placed far from it yet forming another distinct cluster (Fig. 2). Bakhtegan cultivar and most of its crossing progenies also comprise a separate cluster. On the other hand some of the cultivars and their hybrids (for example Color fiber, Sahel, Tashkand, 1106 and 1097) show considerable difference and are placed in different clusters.

PCO plot of cotton cultivars (Fig. 3) also showed the distinctiveness of the cultivars VXSXO, Sahel 77 and hybrids of Chirpan as they form a separate group far from the other cultivars studied. The cultivars C200, 1106 X Sahel, 1097 X Bakhtegan and 1097 also stand far from the other genotypes.

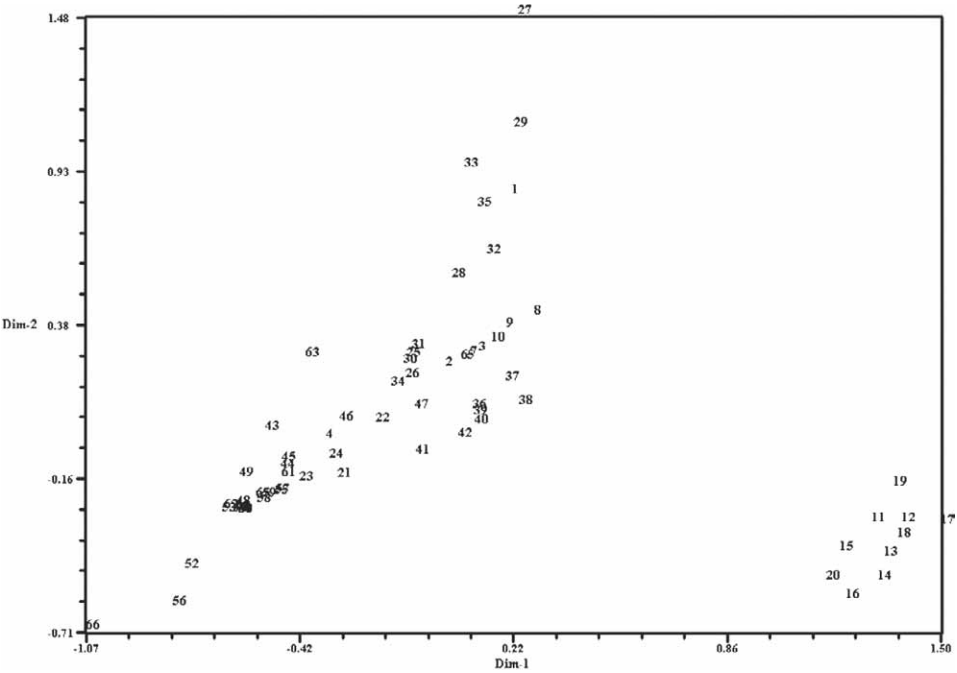

Fig. 3. PC ordination of the cotton cultivars based on cytogenetical data (the cultivars number as in Tab. 1).

Factor analysis of meiotic characters revealed that the first factors comprise about 86% of total variance. In the first factor with about 44% of total variance, meiotic characters including number of rod bivalents and ring bivalents, intercalary chiasmata as well as trivalents possessed the highest positive correlation (>0.65), while the total and terminal chiasmata possessed the highest negative correlation (–0.70 and –0.90 respectively). In the second factor with about 18% of total variance, the number of univalents possessed the highest negative correlation (–0.79), while in the third and forth factors each with about 12 and 11% of total variance, the number of quadrivalents and hexavalents possessed the highest negative (–0.90) and positive (0.90) correlations respectively. Therefore factor analysis

indicates that rod bivalents, ring bivalents, intercalary chiasmata as well as trivalents are the most variable meiotic characters among the cotton cultivars studied.

Discussion

The occurrence of a diffuse stage has been reported in several plant species (SYBENGA 1992). Various reasons have been suggested for the occurrence of a diffuse stage, including high synthetic activity analogous to the lampbrush stage in the amphibian oocyte, meiotic arrest to withstand the adverse environmental conditions, etc. (SHEIDAI and INAMDAR 1991). However at present we do not know the reason for the occurrence of the diffuse stage in cotton.

The significant difference in the chiasma frequency of the cotton cultivars may indicate their genomic differences, as variation in chiasma frequency and localization is genetically controlled and has been reported in several plant species and crop plant varieties. Such a variation in the species/ populations with the same chromosome number is considered a means for generating new forms of recombination influencing the variability within natural populations in an adaptive way (REES and DALE 1974, REES and JONES 1977).

The occurrence of different types of quadrivalents has been considered as an important cytogenetic marker differentiating tetraploid cotton cultivars and also revealing the cultivars' genomic differences (MENZEL and BROWN 1978, ENDRIZZI et al. 1984). The cotton cultivars possessing type I adjacent quadrivalents show high pollen fertility due to the proper chromosomes segregation. Comparatively lower pollen fertility (95.60%) observed in the hybrid cultivar Siokra X 1097 may be due to the occurrence of type II adjacent chromosome orientation, as in this type the chromosomes show duplication/deletion and form unviable gametes (SYBENGA 1992).

V-shaped A-D heterozygote translocations with alternate orientation observed in the cultivars Sahel, Oltan X Sahel and Modified will have proper (balanced) chromosome segregation forming viable gametes. ENDRIZZI et al. (1984) also reported the occurrence of such heterozygote translocations in some of the tetraploid cotton cultivars and considered them a source of new genetic rearrangements in cotton. Similarly, hexavalents formed in a few of cotton cultivars studied are due to the occurrence of heterozygote translocations among 3 pairs of chromosomes, which may increase the amount of genetic recombination in these cultivars.

The chromosome stickiness observed in some of the cultivars may bring about different cytogenetic abnormalities like laggard chromosome formation, multipolar cells and anuploidy. Genetic, environmental factors and their interaction have been considered possible reasons for the occurrence of chromosome stickiness in different plant species and cultivars (BAPTISTA-GIACOMELLI et al. 2000).

Multipolar cells observed in some of the cultivars may also be due to spindle abnormalities. Such meiotic abnormalities may lead to the formation of abnormal tetrads and aneuploid gametes (VILLEUX 1985; NIRMALA and RAO 1996; SHEIDAI and ATTAEI, 2005; SHEIDAI and NOUROOZI 2005; SHEIDAI et al. 2005, 2006).

Migration of chromatin material (cytomixis), which was observed in some of the cotton cultivars, has been considered the cause of aneuploid and polyploid gametes in different plant species and cultivars (FALISTOCCO et al. 1995). Cytomixis occurs through cytoplas-

mic connections originating from the pre-existing systems of plasmodesmata formed within the anther tissues. The plasmodesmata become completely obstructed by the deposition of callose, but in some cases they persist during meiosis and increase in size forming conspicuous inter-meiocytes connections or cytomictic channels that permit the transfer of chromosomes.

The cotton cultivars showing cytomixis also showed different degrees of pollen infertility and abnormal pollen formation, possibly due to unbalanced chromosome migration. The correlation test performed indicated a significant negative correlation between total percentage of cytomixis ($r = –0.78$, $p = 0.01$) and pollen fertility, and also between the percentage of the metaphase cells showing cytomixis ($r = –0.86$, $p = 0.01$) and pollen fertility. Therefore cytomixis may be in part responsible for pollen infertility of the cotton cultivars studied.

Pair-wise χ^2 test performed showed a significant difference in the percentage of cells showing cytomixis among the cultivars studied indicating their genomic differences. This holds true also for the parents and their hybrids, for example the cultivar Sahel 77 differs significantly in the percentage of cytomixis from its crossing progenies i.e. Sahel 77 X Okra and Sahel 77 X 4-S-4.

WEBER (1934) in his study of chromosome number and meiotic behavior in different species and cultivars of *Gossypium* reports the occurrence of aneuploid male gametes in cultivated cotton (he considers aneuploidy a condition due to clumping of chromosomes and lagging bivalents) and raises the question of why aneuploid individuals have not been reported in cotton. He believes that aneuploid individuals either have been overlooked or do not exist due to degeneracy or incompatibility of aneuploid gametes, but as his observations did not show any degeneracy of aneuploid gametes he concludes that aneuploid individuals have been overlooked. The present study indicates that the infertility of aneuploid pollen grains may be partly responsible for aneuploid cotton plants not having been observed.

In some cases cytomixis may lead to the migration of the whole chromatin material among the neighboring meiocytes (Fig. 1, E) and the formation of unreduced gametes (FALISTOCCO et al. 1995; SHEIDAI et al. 1999, 2006). The presence of giant pollen grains has been used as an indication of the production of 2n pollen (VILLEUX 1985). Therefore the larger sized pollen grains in the cultivars C-250, C-200, T-300 and Gukurova may be considered as the potential 2n (unreduced) pollen grains. Identification of the cotton genotypes producing unreduced gametes may be of practical use in the cotton hybridization program.

B-chromosomes are accessory chromosomes reported in many plant and animal species. They show numerical polymorphism and, when present in high numbers, negatively affect the growth and vigor of the plants, while in low numbers they may be beneficial to the plant possessing them (CAMACHO et al. 2000). B-chromosomes may affect the frequency and distribution of chiasmata as well as chromosome association, however due to low number of meiocytes showing presence of B-chromosomes in tetraploid cotton cultivars studied, their effects on chiasma frequency and chromosome associations could not be worked out except in the cultivar Gukurova. T-test analysis revealed that the presence of B-chromosomes significantly increased the mean values of terminal and total chiasmata as well as amount of quadrivalents in the cells possessing B-chromosomes compared to those devoid of Bs. An increase in chiasma number may bring about more genetic

variation for a plant possessing B-chromosomes and increase in the number of quadrivalents may create new linkage group among the genes, which in turn increase genetic variability of the cells.

As chiasma frequency and localization is genetically controlled (REES and DALE 1974, REES and JONES 1977), grouping of the cultivars based on these characteristics may partly indicate the genetic relationship of the cultivars studied. This is supported by different clusters/groups obtained (Fig. 2), for example the Oltan cultivar and its crossing progenies as well as the Bakhtegan cultivar and its crossing progenies have been placed in a single cluster close to each other.

ANOVA as well as clustering and PCO ordination revealed cytogenetic distinctiveness of the cotton cultivars studied. Accordingly, if such cytogenetic variations are combined with the morpho-agronomic characteristics, a better hybridization program may be planned for the breeding of cotton in Iran.

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