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CHROMOSOME PAIRING AND HETEROZYGOTE TRANSLOCATION IN OLTAN COTTON CULTIVAR AND ITS CROSSING PROGENIES*

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Abstract – A cytogenetic study was performed on 11 tetraploid cotton cultivars (*Gossypium hirsutum* L.) including the Oltan cultivar and its crossing progenies. The chromosome pairing and chiasma frequency, as well as meiotic abnormalities were compared among the genotypes studied. Heterozygote translocations with alternate orientation were observed between some of the chromosomes of the A genome and those of D genome in some of the cultivars, which may bring about new genetic rearrangement to be used in breeding programs. Meiotic abnormalities including formation of laggard chromosome, stickiness, as well as disorganized chromosomes occurred in some of the cultivars, which may cause pollen sterility. B-chromosomes were observed in some of the cultivars. The cultivars studied differed significantly in their cytogenetic characteristics, partly indicating their genomic differences, which may be used in cotton breeding.

Keywords – B-Chromosome, cotton, cytogenetic, heterozygote translocation

1. INTRODUCTION

Tetraploid cotton (*Gossypium hirsutum*) with the genome constitution 2 $(AD)_1$ (2n=52), along with *G. barbadense* dominate world cotton production [1]. 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. The chromosomes of the genome A are relatively bigger than those of the D genome, and pairing takes place within members of each genome only [2, 3].

Cotton is considered one of the most important crop plants in Iran. Different cultivars of *G. hirsutum* and *G. herbaceum* are extensively cultivated in various regions of the country in general, and in northern regions of Iran such as the Gorgan and Gonbad areas in particular.

In Iran, our earlier studies on cotton considered morphometric, karyotypic, as well as meiotic analysis of some tetraploid cotton cultivars [4, 5].

Continuous cultivation of the same genotypes may bring about genetic erosion in the long term, as such, study of the available genetic variability, as well as introducing new ones, is of importance. Therefore hybridization was carried out among available tetraploid cotton cultivars having some specific morphological and agronomic characteristics, as well as resistance to *Verticilium* wilt [6] and their cytogenetic characteristics were studied.

2. MATERIALS AND METHODS

a) Experimental lines

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In total 11 genotypes were used in cytogenetic studies (Table 1) obtained from crossing the Oltan cultivar with 5 other tetraploid cotton cultivars, namely Sahel, Zeta2, B557, Tashkand1, and Modified. The plants were cultivated in three rows of 10 m length with a 20 cm interplant distance in the experimental field of the Varamin Cotton Research Center of Iran, according to a completely randomized design (CRD) with 3 replications.

Table 1. Meiotic characteristics in cotton cultivars studied

Cultivar	TX	IX	TOX	Q	U	RB	ROD
OxZ	38.66	2.33	41.00	.00	3.20	16.33	8.00
OxT	26.44	1.55	28.00	.00	19.32	10.88	5.40
OxM	34.40	2.30	36.70	.00	7.20	13.20	9.20
OxS	27.58	2.33	29.91	.33	13.32	10.33	8.30
Zeta2	39.21	3.73	42.89	.00	5.14	17.00	6.42
Tashkand	38.28	3.21	41.50	.00	5.28	16.07	7.28
Modified	31.58	4.00	35.58	.00	8.50	13.30	8.41
Sahel	32.27	2.90	35.18	.09	13.44	13.63	5.45
Oltan	39.78	2.78	42.57	.21	3.46	16.78	7.05
OxB	31.50	2.16	33.66	.08	10.16	12.25	8.50
B557	35.30	2.50	37.80	.00	7.00	14.40	8.10

TX=Terminal chiasmata, IX=Intercalary chiasmata, TOX=Total chiasmata,
Q=Quadrivalent, U=Univalent, RB=Ring bivalent, ROD=Rod bivalent
O=Oltan, T=Tashkand, M=Modified, S=Sahel, B=B557, Z=Zeta2

b) Cytogenetic studies

For cytogenetical studies, 10 lines belonging to 11 genotypes were used. Fifty flower buds were collected randomly from 10 randomly selected plants of 10 lines from each genotype making the total collection in each genotype to be 50×10×10=5000. The flower buds were fixed in ethanol: glacial acetic acid (3:1) for about 24 hr. The flower buds were then preserved in 75% ethanol at 4^{o} C until used. The squash technique was employed for meiotic preparation using 2% acetocarmine [3]. Meiotic analyses were performed using 500 prophase-I, 50-100 metaphase-I and II cells, 500 anaphase I and II cells as well as 500 telophase I & II cells in each genotype. The frequency and distribution of chiasmata were recorded at metaphase and diplotene stages of meiosis I.

c) Statistical analyses

Whether the differences observed in cytogenetic characters are significant or not, statistical tests were used that included the analysis of variance (ANOVA) performed among the genotypes followed by the least significant difference test (LSD) [4].

For grouping the cotton lines showing similar meiotic characteristics, different clustering methods including single linkage, UPGMA and WARD, as well as ordination based on principal components analysis (PCA) was performed [3]. For cluster analysis, standardized data (mean=0, variance=1) were used [4]. Statistical analysis used SPSS ver.9 (1998).

3. RESULTS AND DISCUSSION

The results of cytogenetic analyses are presented in Table 1 and Figs. 1-4. The meiotic analysis of all 11 cotton lines studied showed a deviant course of prophase in meiosis-I as despiralization of the chromosomes occurred after pachytene entering the diffuse stage (Fig. 1). The occurrence of the diffuse stage has been reported in several plant species [4, 7], which may be complete in which the whole chromosomes decondense or only some parts of the genome show decondensation. The present study

showed the occurrence of partial to complete decondensation in both parental lines and their hybrids.

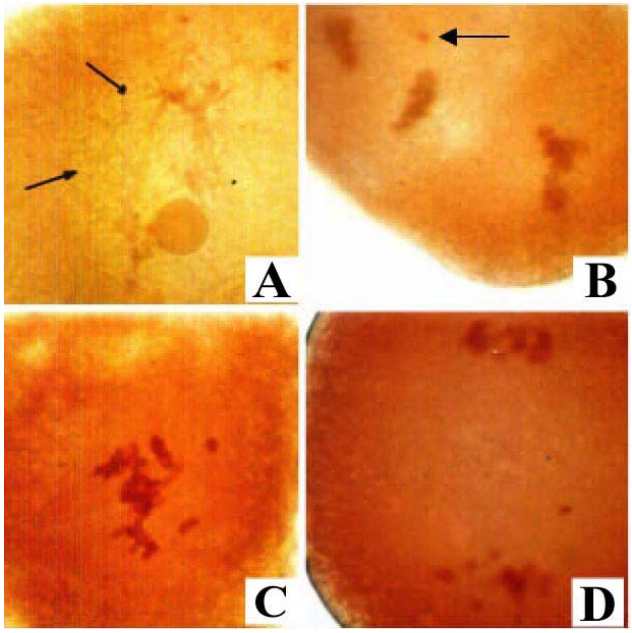

Fig. 1. Representative meiotic cells in cotton cultivars studied, A=Diffuse stage in cultivar Oltan X B557 (arrows indicate decondensed regions), B=Laggard chromosome (arrow) in telophase-II cell in the cultivar Oltan X B557, C=Chromosome stickiness in the cultivar Oltan X B557, D=Laggard chromosome in anaphase-I cell Oltan X Sahel

Various reasons have been suggested for the occurrence of a diffuse stage, which are: high synthetic activity analogous to the lampbrush stage in amphibian oocyte, shedding of the lateral elements in the synaptonemal complex, the post pachytene elimination or modification of histone proteins and meiotic arrest to withstand the adverse environmental conditions [8]. At present we do not know the reason for the occurrence of a diffuse stage in the cotton cultivars studied.

Data with regard to chiasma frequency and distribution, as well as chromosome pairing is presented in Table 1. All cotton cultivars studied possessed 2n=4x=56 (n=23) chromosome number and mostly formed bivalents in metaphase-I, except a low value of quadrivalent formation in 4 cultivars of Sahel, Oltan, Oltan×Sahel and Oltan×B557 (Table 1).

The cultivars Zeta2 possessed the highest values of ring bivalents (17.00), while hybrid cultivar Oltan×B557 possessed the lowest value (12.25). The highest value of rod bivalents occurred in the hybrid cultivar Oltan×Modified (9.20), while the lowest value occurred in Oltan×Tashkand1 (5.40).

Adjacent and alternate quadrivalents were formed in the cultivars Sahel, Oltan, Oltan×B557 and Oltan×Sahel (Table 1, Fig. 2). An interesting observation was the occurrence of heterozgote translocations (A-D) with alternate orientation in metaphase-I in some of the cultivars (Fig. 2), which may lead to the formation of viable gametes and bring about new genetic rearrangements in cotton cultivars.

Endrizzi et al. [1] reported the occurrence of V-shaped heterozgote translocation with alternate orientation, which was also observed in cotton lines studied here (Fig. 2). Various types of quadrivalents are considered as important cytogenetic markesr differentiating different cotton cultivars [1]. This is the first report of V-shaped heterozgote translocation in cotton cultivars available in Iran.

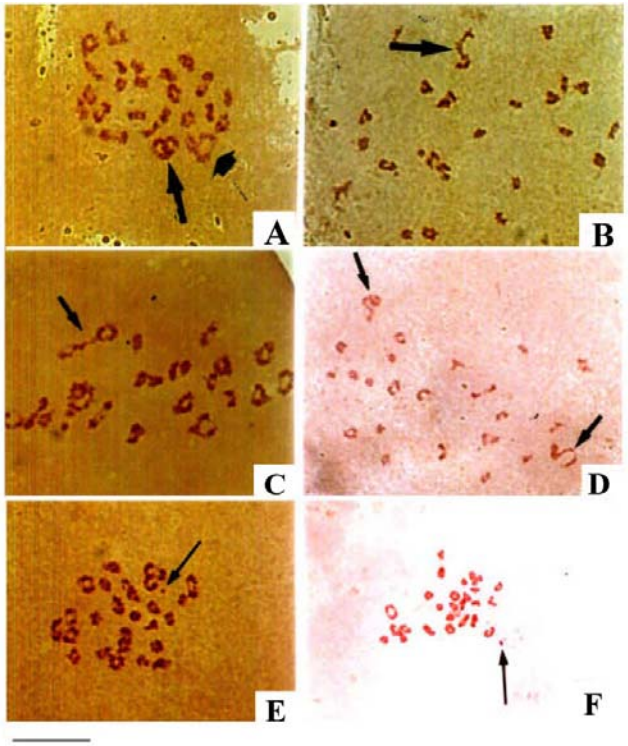

Fig. 2. Representative meiotic cells in cotton cultivars studied (scale bar=10 µm), A=Alternate quadrivalent (arrow) and heterozygote translocation (arrow head) in the cultivar Oltan X Sahel, B=Heterozygote translocation (arrow) in the cultivar Sahel, C=Alternate quadrivalent (arrow) in the cultivar Sahel, D=Heterozygote translocation (arrow) in the cultivar Modified, E=B-chromosome (arrow) in the cultivar Sahel, F=B-chromosome (arrow) in the cultivar Oltan X Sahel

The highest value of total chiasmata occurred in cultivar Zeta2 and Oltan cultivars (42.89 and 42.57 respectively), while the lowest value occurred in the hybrid Oltan×Tashkand1 (28.00). The highest value of terminal chiasmata occurred in the Oltan cultivar (39.78), while the lowest value occurred in hybrid Oltan×Tashkand1 (26.44). However this hybrid cultivar possessed the lowest value of intercalary chiasmata (1.55). The highest value of intercalary chiasmata occurred in the Modified cultivar (4.00). ANOVA and LSD tests performed on chromosome pairing and chiasma frequency revealed a significant difference among the cultivars studied for almost all meiotic characters.

Variation in chiasma frequency and localization is genetically controlled and has been reported in several plant species as well as in crop plant varieties [9]. Such a variation in the species/ populations with the same chromosome number is considered as a means for generating new forms of recombination influencing the variability within natural populations in an adaptive way [10]. Therefore since the cultivars studied were grown under uniform conditions, the presence of a significant difference in their meiotic characteristics may partly indicate their genomic differences.

Some meiotic abnormalities were observed in some of the cultivars such as chromosome stickiness in metaphase and anaphase, disorganized chromosome, i.e. chromosome/s not aligned with the others on the equator, laggard chromosomes in anaphase I and II and micronuclei in telophase I and II (Fig. 1).

Chromosome stickiness occurred in all cultivars studied barring Zeta2, Sahel and Oltan×Sahel. The degree of chromosome stickiness ranged from stickiness among two or more chromosomes to the involvement of all metaphase chromosomes forming a complete clump (Fig. 1). Genetic and environmental factors, as well as their interaction have been considered for the occurrence of chromosomes stickiness [11, 12].

Laggard chromosomes occurring in anaphase I and II as well as telophase-I and II, ranging from 1 to a few chromosomes (Fig. 1) were observed in Zeta2, Tashkand, Modified, Oltan, Oltan×Zeta2, Oltan×Modified, Oltan×Sahel and Oltan×B557.

B-chromosomes

Among the cotton cultivars chromosomes studied, 0-2 B-chromosomes (Bs) were observed in Sahel, Tashkand, Oltan×Tashkand, Oltan×Zeta2 and Oltan×Sahel (Fig. 2). 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 cotton lines studied also supports transmission of these chromosomes from parents to the next generation.

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 [13, 14]. B-Chromosomes may affect the frequency and distribution of chiasmata as well as chromosome association**,** either directly or by affecting the genes present on the A-chromosomes that control meiosis [14].

Recently B-chromosomes were reported for the first time in an imported tetraploid cotton cultivar [15] which could significantly increase the mean values of terminal and total chiasmata, as well as the amount of quadrivalents in the cells possessing B-chromosomes compared to those devoid of Bs. Therefore, the present study reports for the first time the occurrence of B-chromosomes in domesticated cotton cultivars of Iran. However, due to a low number of metaphase cells possessing B-chromosomes and difficulties in identifying them among 26 bivalents in the tetraploid cotton cultivars studied, the effect of B-chromosomes on chiasma frequency and distribution could not be studied and needs further investigation.

In order to determine the cultivars having cytogenetic similarities, different methods of cluster analysis as well as ordination based principal components analysis (PCA) was performed which produced similar results (Figs. 3 & 4).

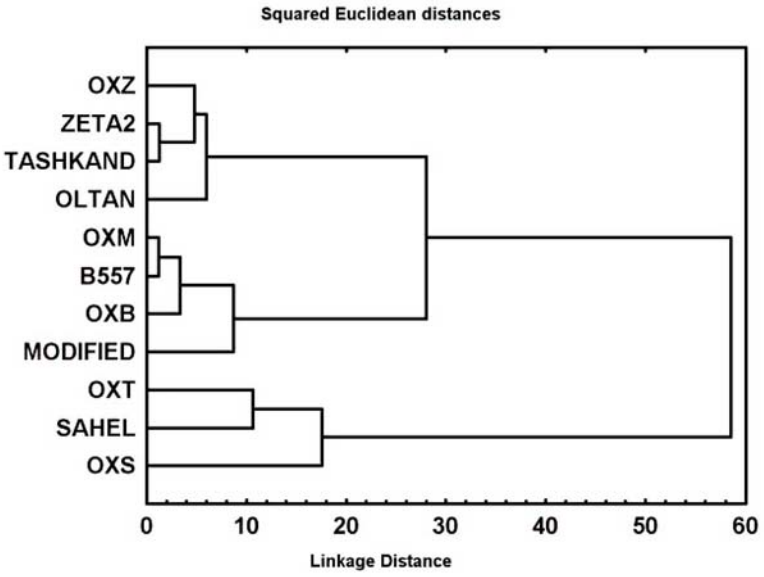

Fig. 3. WARD cluster analysis and PCA ordination of cotton cultivars. (Cultivars name as in Table 1)

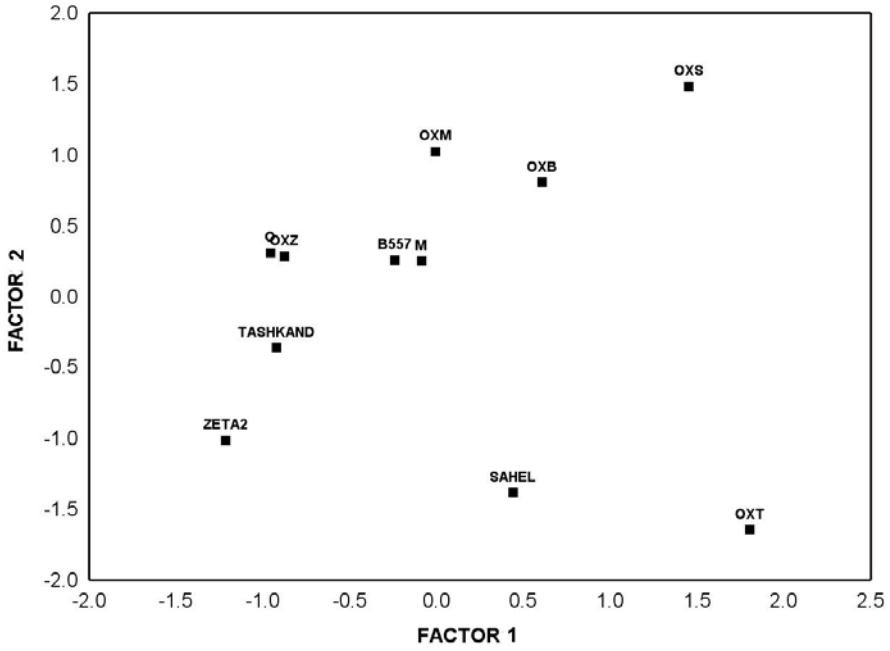

Fig. 4. PCA ordination of cotton cultivars. (Cultivars name as in Table 1)

In general, 3 major clusters/ groups are formed. The first major cluster/ group is comprised of B557, Modified and hybrid cultivars Oltan×B557 and Oltan×Modified. Therefore it seems that the effect of B557 and Modified genotypes is more on cytogenetic characteristics of their hybrid compared to that of Oltan as they are placed in one cluster close to these parental cultivars.

The second major cluster is comprised of Oltan, Zeta 2, Tashkand and hybrid cultivar Oltan×Zeta 2, while the third major cluster is comprised of Sahel and two hybrid cultivars, Oltan×Sahel and Oltan×Tashkand. The members of this cluster are placed some distance from the other cultivars.

Factor analysis of meiotic characters revealed that the first two factors comprise about 77% of the total variance. In the first factor, with about 60% of total variance, meiotic characters including the number of ring bivalents, total and terminal chiasmata possessed the highest positive correlation (>0.90). This factor separates mainly Oltan×B557 and Sahel cultivars from the other cultivars (Fig. 4). Factor two comprises about 10% of total variance in which the number of rod bivalents possessed the highest positive correlation (>0.90). This factor separates Oltan×B557, Oltan×Sahel and Modified cultivars from the others. Therefore these are the most variable meiotic characters among the irradiated cotton cultivars studied.

The present findings, if combined with agronomic characteristics of the cultivars studied, may be used in planning cotton hybridization and breeding programs.

REFERENCES

1. Endrizzi, J. E., Turcotte, E. L. & Kohel, R. J. (1984). *Qualitative Genetics, Cytology and Cytogenetics*. Cotton, Agronomy Monograph, No. 24, Madison.
2. Menzel, M. Y. & Brown, M. S. (1978). Genetic lengths and break points in twelve chromosomes of *Gossypium hirsutum* involved in ten reciprocal translocations. *Genetics*, *88*, 541-558.
3. Menzel, M. Y., Brown, M. S. & Naqi, S. (1978). Incipient genome differentiation in *Gossypium*. 1. Chromosomes 14, 15, 16, 19 and 20 assessed in *G. hirsutum*, *G. raimondi* and *G. lobatum* by means of seven A-

D translocations. *Genetics*, *90*, 133-149.

4. Sheidai, M., Vafai-Tabar, M., Mirzai-Nedoshan, H. & Hosseini-Nejad, Z. (1998). Cytogenetical studies in *Gossypium hirsutum* L. cultivars and their hybrids. *Cytologia 63*, 41-48.
5. Sheidai, M., Azarani, H. & Hosseynine-Nejad, Z. (2002). Cytogenetic study of Gamma irradiated lines of cotton (*Gossypium hirsatum* L.). *I. R. J. Sci.13*, 311-322.
6. Nemati, N., Hosseininejad, Z. & Rastegari, S. J. (1995). The use of mutation (Cobalt 60) in production of new resistant cotton lines against *Verticilium* wilt. Final report. Cotton Research Center, Varamin.
7. Sybenga, J. (1992). *Cytogenetics in Plant Breeding*. Spring-Verlag, Berlin, Germany.
8. Sheidai, M. & Inamdar, A. C. (1991). Variations in meiotic chromosomes in *Asparagus* L. *Biovigyanum, 17*, 45-47.
9. Rees, H. & Jones, R. N. (1977). *Chromosome Genetics.* London, Edward Arnold.
10. Rees, H. & Dale, P. J. (1974). Chiasma and variability in *Lolium* and *Festuca* populations. *Chromosoma*, *47*, 335-351.
11. Nirmala, A. & Rao, P. N. (1996). Genetics of chromosome numerical mosaism in higher plants. *The nucleus*, *39*, 151-175.
12. Baptista-Giacomelli, F. R., Pagliarini, M. S. & Almeida, J. L. (2000). Meiotic behavior in several Brazilian oat cultivars (*Avena sativa* L.). *Cytologia*, *65*, 371-378.
13. Clark, M. S. & Wall, W. J. (1996). *Chromosomes The Complex Code*. London, Chapman & Hall.
14. Camacho, J. P. M., Sharbel, T. F. & Beukeboom, L. W. (2000). B-chromosome evolution. Phil. Trans. R. Soc. Lond. B, *355*, 163-178.
15. Sheidai, M. & Roknizadeh, H. (2004). B-chromosomes, cytomixis and unreduced gametes in *Gossypium hirsutum*. The nucleus (in press).