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CYTOGENETIC STUDY OF GAMMA IRRADIATED LINES OF COTTON (*GOSSYPIUM HIRSUTUM* L.)

M. Sheidai[1,*], H. Azarani[2] and Z. Hosseininejad[3]

[1] *Department of Biology, Shahid Beheshti University, Evin, Tehran, Islamic Republic of Iran*
[2] *Department of Genetic, Azad University, Tehran, Islamic Republic of Iran*
[3] *Cotton Research Center, Varamin, Islamic Republic of Iran*

Abstract

A cytogenetic study was performed on M4 generation of gamma irradiated lines of Shirpan cultivar of tetraploid cotton (*Gossypium hirsutum* L.). A significant difference was observed in cytogenetical characteristics such as the number and type of quadrivalents as well as percentage of cells showing abnormality among the irradiated and non irradiated lines. Cytomixis and post-pachytene diffuse stage was observed in the gamma irradiated lines, but not in the control line. There was an indication that the lines with good agronomic characters possess meiosis with a low abnormality and a higher value of alternate quadrivalents.

Keywords: *Gossypium hirsutum*; Shirpan cultivar; Cytogenetics; Cluster analysis; Principal components analysis; Gamma irradiation

Introduction

Upland cotton (*Gossypium hirsutum*) is an allotetraploid species of genome constitution 2 $(AD)_1$ (2n=52), produced from hybridization of *G. herbaceum* (2n=26, A_1)×*G. thurberri* (2n=26, D_1; 9). *G. hirsutum* along with *G. barbadense* now dominate world cotton production [3]. Cultivars of *G. hirsutum* have shorter and weaker fibers, but usually produce higher yield than *G. barbadense*. 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 genome D and pairing takes place within members of each genome only [10].

Mutation breeding has been used for improving both oligogenic and polygenic characters, improving morphological and physiological characters, disease resistance, and quantitative characters including yielding ability. Mutagenesis may induce desirable mutant alleles, which may not be previously present in the germplasm. Mutation breeding relieves the complete dependence of breeders on the natural germplasm, but it should be remembered that mutation breeding can not minimize the necessity of germplasm collections; it only serves as a useful supplement to the available germplasm [24]. Mutation breeding using gamma irradiation has been used extensively in upland cotton leading to improvement in quantitative characters, insensitivity against the photoperiod, earliness, yield and oil content. Such studies have shown the occurrence of cytogenetical abnormalities and phenotypic changes in mutants [1,6,8,9,15,16].

More than 337 varieties including cereals, oilseeds

* *E-mail: msheidai@cc.sbu.ac.ir*

(such as cotton), pulses, millets, vegetables and fruits have been produced as a result of mutagenesis programs in different countries of the world [23]. In many instances mutation breeding using ionizing radiation (including gamma rays) has led to phenotypic and cytogenetic abnormalities [23].

Cotton is considered as one of the most important crop plants in Iran, cultivated in various regions of country but mainly is grown in northern regions of Iran such as Gorgan and Gonbad areas. Different cultivars of *G. hirsutum* and *G. herbaceum* are extensively cultivated in Iran. 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. Therefore Gamma irradiation of tetraploid cotton (Shirpan) was originally carried out with the aim to induce new genetic variability in the upland cotton of Iran which led to the production of some mutant lines with specific morphological and agronomic characteristics as well as resistance to *Verticilium* wilt [12].

The present report considers cytogenetic characteristics of gamma irradiated lines of Shirpan cultivar in order to study the effects of gamma irradiation on meiotic cells compared to that of control line and also if possible correlate any particular cytogenetic features with morphological and agronomic characteristics. Our earlier studies on cotton considered morphometric, karyotypic as well as meiotic analysis of some cultivars and their hybrids [21-23].

Materials and Methods

Experimental Lines

Originally, seeds of Shirpan cultivar were treated with 150, 200, 250, 300 and 350 gray (Gy) of gamma rays using Cobalt 60. Plants were raised from M1 to M4 by selfing and selection was performed based on important agronomic characters like earliness, lint quality and yield [12].

The present report considers cytogenetic analysis of ten lines including Shirpan (*G. hirsutum*) as the control and nine gamma irradiated lines of M4 generation treated with the lowest and highest doses of 150 and 350 gray (Gy), showing some phenotypic and agronomic peculiarities [12].

The experimental lines studied, are presented in Table 2, the names with C15 and C35 indicate treatment with 150 and 350 Gy. The plants were cultivated in 3 rows of 10 m length with 20 cm interplant distance, in the experimental field of Varamin Cotton Research Center of Iran, according to a completely randomized design (CRD) with 3 replications.

Cytogenetic Studies

For cytogenetical studies, 50 flower buds were used randomly from 10 randomly selected plants of each line making the total collection 50×10×10=5000. The flower buds were fixed in ethanol (70%) and glacial acetic acid (3:1) for about 24 h. The flower buds were then preserved in 75% ethanol at 4°C until used. Squash technique was employed for meiotic preparation using 2% acetocarmine as the stain [20,23]. Meiotic analyses were performed using 50-100 metaphase-I & II cells, 500 anaphase-I & II cells and 500 telophase-I & II cells.

In total, 26 meiotic characteristics were used in cytogenetic analysis (Table 1). Chiasma frequency and distribution as well as association of chromosomes were determined using 50 pollen mother cells (PMCs). Chromosome segregation during anaphase and telophase was studied in about 500 PMCs. Pollen stainability as a measure of fertility was checked by staining the pollen grains with 2% acetocarmine and 50% glycerin for 1/2 h. Round/completely stained pollen grains were considered fertile and unstained/ shrunken grains as infertile [17].

Table 1. Cytogenetical characters studied

1- Metaphase-I cells showing abnormality (%)
2- Anaphase-I cells showing abnormality (%)
3- Telophase-I cells showing abnormality (%)
4- Metaphase-II cells showing abnormality (%)
5- Anaphase-II cells showing abnormality (%)
6- Telophase-II cells showing abnormality (%)
7- Cells with unequal segregation
8- Cells with cytomixis
9- Cells with stickiness
10- Cells with disorganized chromosomes
11- Cells with laggard chromosomes
12- Chromosomes with two chiasmata
13- Chromosomes with one chiasma
14- Chromosomes with no chiasma
15- Intercalary chiasma/cell
16- Terminal chiasma/cell
17- Total chiasma/cell
18- Cells with two univalents
19- Cells with two trivalents
20- Cells with one quadrivalent
21- Cells with two quadrivalents
22- Cells with three quadrivalents
23- Adjacent quadrivalent
24- Alternate quadrivalent
25- Ring bivalents
26- Rod bivalents

Table 2. Genotypes and their cytogenetical characteristics. Characters number as in Table 1

Genotype	Cytogenetic characters																
	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
C15-1	1.00	0.00	3.33	1.67	8.33	16.67	9.00	0.33	11.83	19.00	30.83	1.00	0.00	1.50	26.67	15.00	0.00
C15-2	1.00	2.00	14.16	16.81	13.46	15.70	9.80	0.50	13.50	17.80	31.30	4.00	0.00	0.00	10.00	10.00	0.00
C15-3	0.00	1.00	13.49	6.35	29.91	17.00	8.78	0.22	15.00	19.00	34.00	2.00	0.00	0.00	24.35	3.00	0.00
C15-4	0.00	0.00	3.26	30.77	25.69	17.58	8.32	0.11	16.39	19.83	36.22	1.00	0.00	1.50	32.31	6.00	0.00
C15-5	0.00	1.00	4.81	16.35	19.86	18.35	7.53	0.12	12.13	20.93	33.07	1.00	0.00	1.00	13.13	1.00	0.00
C15-8	2.00	5.00	25.83	18.33	4.90	19.44	6.38	0.19	16.06	21.25	37.32	1.00	0.00	2.00	14.43	3.00	1.00
C35-9	0.00	0.00	2.94	3.92	20.00	14.83	11.00	0.17	15.39	15.85	31.23	3.00	0.00	0.00	20.00	4.50	0.00
C35-10	0.00	6.00	7.41	14.81	23.78	17.16	8.58	0.26	14.06	17.39	31.44	9.00	1.00	2.00	16.67	0.00	0.00
C35-11	0.00	2.00	12.00	9.33	50.00	15.10	10.55	0.35	10.25	17.90	28.15	8.00	0.00	2.50	8.10	1.00	0.00
Control	0.00	0.00	8.45	19.72	7.09	19.91	6.09	0.00	13.55	21.64	35.18	0.00	0.00	0.00	6.00	0.00	0.00

Statistical Analyses

In order to determine a significant difference in chiasma frequency, meiotic abnormalities and number of quadrivalents, a pair-wise χ^2 test was performed among the lines studied [20]. For grouping the lines showing similar meiotic characteristics, different clustering methods as well as ordination based on principal components analysis (PCA) were performed [2,21]. Statistical analysis was performed using SPSS ver. 9 (1998).

Results and Discussion

The cytogenetic features of the cotton lines investigated are depicted in Table 1, Figures 1-3. Details of meiotic characters including chiasma frequency and distribution as well as association of chromosomes are presented in Table 2, while frequency of alternate and adjacent quadrivalents are given in Figure 2. The percentage of metaphase, anaphase and telophase cells showing meiotic abnormality are given in Figure 3.

The following meiotic characteristics was considered abnormal: 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, micronuclei in telophase-I and II, unequal segregation of chromosomes during anaphase and occurrence of cytomixis. Details of meiotic abnormalities are presented in Table 2 and Figure 3.

The highest values for total chiasmata were observed in the line C15-8 (37.32) followed by the line C15-4 (36.22), while the lowest value occurred in C35-11 (28.15). Shirpan and C15-8 possessed the highest values of the terminal chiasmata (21.64 and 21.25 respectively) among the lines studied (Table 2), while C35-9 possessed the lowest value (15.85). C15-4 and C15-8 possessed the highest values of the intercalary chiasmata (16.39 and 16.06 respectively), while C35-11 possessed the lowest value (10.25).

Variation in chiasma frequency and localization is genetically controlled [13] and has been reported in several wild as well as cultivated plant species [14,22]. Pair-wise χ^2 test performed did not show any significant difference in chiasma frequency among the lines indicating that a minor change in the genes controlling chiasma formation and distribution has been occurred. Such a variation in species or 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 [13].

It is interesting to mention that this small change in chiasma frequency and distribution among the cotton lines studied, has led to a significant change in the number and types of quadrivalent (adjacent & alternate), which is considered important in tetraploid cotton lines for producing genetic variation or rearrangement [3,10,11].

The highest value of ring bivalents occurred in Shirpan and C15-8 (19.90 and 19.40 respectively) while the lowest value occurred in C35-9 (14.80). The highest value of rod bivalents occurred in C35-11 and C15-2 (10.90 and 10.30 respectively), while the lowest value occurred in Shirpan and C15-8 (6.10 and 6.60 respectively).

With regard to meiotic abnormalities, the highest

Table 3. Pearson coefficient of correlation (r) among meiotic characters and pollen fertility. R > 0.60 is significant at p = 0.05. Abbreviations: PO = Pollen fertility, 1- 26 = Meiotic characters as in Table 1 (character 21 is not included as it was not observed in the C-15 lines studied)

PO	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
	1.00																									
1	-.14	1.00																								
2	.02	.11	1.00																							
3	.29	-.19	.61	1.00																						
4	-.04	.31	.05	-.29	1.00																					
5	.54	-.03	-.18	-.19	-.00	1.00																				
6	-.82	.27	-.24	-.57	.20	-.11	1.00																			
7	-.47	.47	.22	-.56	.34	-.02	.66	1.00																		
8	-.58	.66	.13	-.13	.62	-.36	.52	.37	1.00																	
9	-.24	.87	.38	-.18	.40	.05	.44	.76	.63	1.00																
10	.13	.31	-.12	-.12	-.08	.64	.02	-.07	.09	.18	1.00															
11	.20	-.12	.63	.92	-.19	-.33	-.62	-.57	.02	-.20	-.05	1.00														
12	-.29	.42	-.52	-.50	-.27	.38	.57	.23	.15	.30	.55	-.57	1.00													
13	.31	-.45	.51	.53	.25	-.36	-.58	-.28	-.18	-.34	-.55	.58	-1.00	1.00												
14	-.06	.16	.50	-.03	.47	-.50	-.21	.35	.26	.23	-.40	.22	-.70	.61	1.00											
15	-.03	-.04	-.18	-.31	.42	.66	.43	.23	.14	.16	.50	-.43	.30	-.27	-.39	1.00										
16	-.22	.39	-.31	-.36	-.54	.33	.40	.26	-.06	.30	.54	-.43	.90	-.92	-.55	.07	1.00									
17	-.17	.24	-.33	-.46	-.07	.68	.57	.34	.06	.31	.71	-.59	.80	-.80	-.64	.74	.72	1.00								
18	-.29	.42	-.52	-.50	-.27	.38	.57	.23	.15	.30	.55	-.57	1.00	-1.00	-.66	.29	.91	.82	1.00							
19	.29	-.42	.52	.50	.27	-.38	-.57	-.23	-.15	-.30	-.55	.57	-1.00	1.00	.66	-.29	-.91	-.82	-1.00	1.00						
20	-.06	.16	.40	.47	.46	-.54	-.29	-.28	.57	.05	-.24	.68	-.60	.55	.53	-.33	-.66	-.67	-.60	.60	1.00					
22	-.68	.17	.55	.35	-.13	-.49	.30	.15	.53	.27	.01	.48	.00	-.02	.16	-.21	.07	-.10	.00	-.00	.44	1.00				
23	-.36	.09	.22	-.57	.27	-.11	.40	.85	.08	.37	-.17	-.48	-.10	.04	.59	.10	.03	.08	-.10	.10	-.27	.04	1.00			
24	.12	-.55	.07	-.26	.00	.20	-.15	.15	-.49	-.36	.05	-.18	-.40	.35	.32	.23	-.21	.02	-.40	.40	-.35	-.27	.60	1.00		
25	-.32	-.73	.02	-.09	-.11	.11	.22	.00	-.25	-.47	.08	-.07	-.10	.15	-.10	.43	-.14	.20	-.10	.10	-.25	.18	.29	.70	1.00	
26	-.08	-.42	.11	-.43	.06	-.06	.03	.44	-.36	-.17	-.32	-.34	-.30	.31	.53	-.04	-.20	-.16	-.30	.30	-.29	-.16	.82	.84	.52	1.00

value of metaphase-I abnormality was observed in C15-8 and C15-2 (Fig. 3), while the lowest values occurred in C15-1, C15-4 and C35-9. These lines possessed the lowest values of abnormality in metaphase-II. The highest value of anaphase-I abnormality was observed in C15-11 while the lowest values occurred in Shirpan and C15-5 (Fig. 3). Pair-wise χ^2 test performed showed a significant difference in the percentage of cells showing meiotic abnormalities.

C15-8 also possessed a high value of unequal segregation and chromosome stickiness (Table 2). Sticky chromosomes were observed from metaphase-I till telophase-II stages in all cotton lines studied (Fig. 1, Table 2). Genetic as well as environmental factors have been considered as the reason for chromosome stickiness [5]. The percentage of cells showing stickiness differed significantly among the cotton lines studied, which may also indicate the genomic differences among the lines studied.

Percentage of pollen fertility is presented in Figure 4. Shirpan possessed the highest percentage of pollen fertility followed by C15-2 and C35-9. T-test analysis did not show a significant difference in pollen fertility between lines treated with 150 and 350 Gy.

Pollen fertility showed a negative significant correlation with telophase-II cells showing meiotic abnormality as well as metaphase cells having 3 quadrivalents ($r=-0.60$, $p<0.05$, Table 3). It also showed a nearly significant correlation with cytomixis ($r=-0.58$). Therefore such meiotic abnormalities may be partly responsible for reduction in pollen fertility in cotton lines studied.

Cluster analysis and ordination of the lines irradiated with 150 grays are presented in Figures. 5 and 6. Both analyses produced similar results. C15-5 showed similarity to Shirpan (control line) in its meiotic characteristics. C15-1 shows similarity to C15-4 and C15-2 to C15-3, but C15-8 due to difference in its meiotic characteristics stands in a separate cluster.

Factor analysis of meiotic characters revealed that the first four factors comprise about 76% of total variance (Table 4). In the first factor with about 32% of total variance, meiotic characters including number of rod bivalents, bivalents with one chiasma, mean number of chromosomes with no chiasma, number of adjacent and alternate quadrivalents possessed the highest correlation (>0.70, Table 4). This factor separates Shirpan, C15-5 and C15-8 from the other cotton lines (Fig. 6).

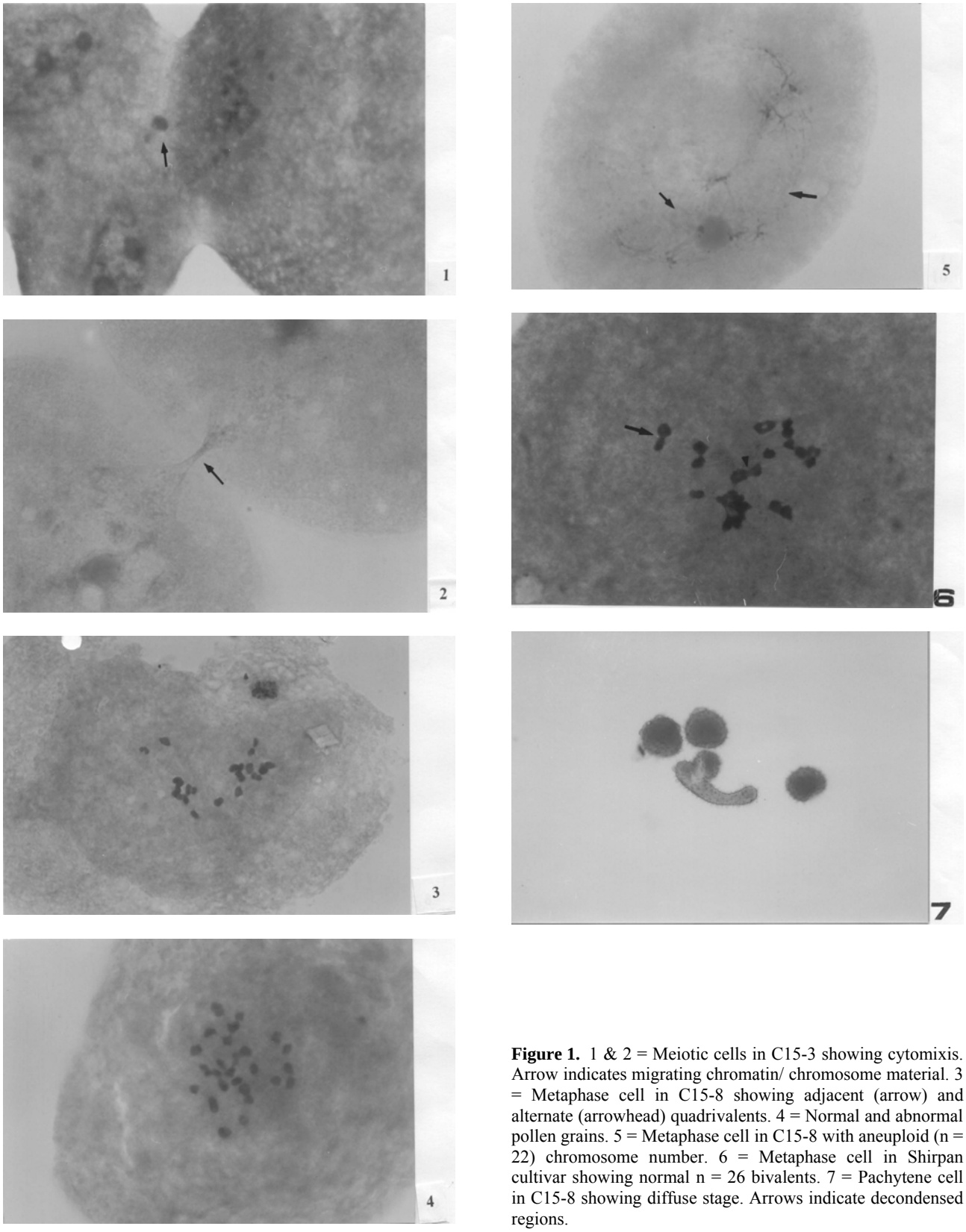


Figure 1. 1 & 2 = Meiotic cells in C15-3 showing cytomixis. Arrow indicates migrating chromatin/ chromosome material. 3 = Metaphase cell in C15-8 showing adjacent (arrow) and alternate (arrowhead) quadrivalents. 4 = Normal and abnormal pollen grains. 5 = Metaphase cell in C15-8 with aneuploid (n = 22) chromosome number. 6 = Metaphase cell in Shirpan cultivar showing normal n = 26 bivalents. 7 = Pachytene cell in C15-8 showing diffuse stage. Arrows indicate decondensed regions.

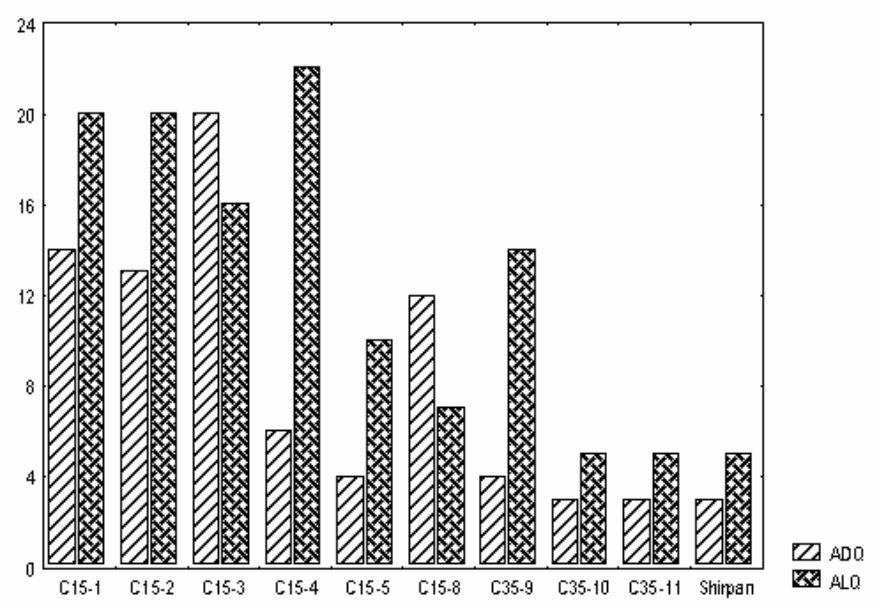


Figure 2. Mean frequency of adjacent and alternate quadrivalents in cotton lines. Abbreviations: ADQ = Adjacent quadrivalent, ALQ = Alternate quadrivalent.

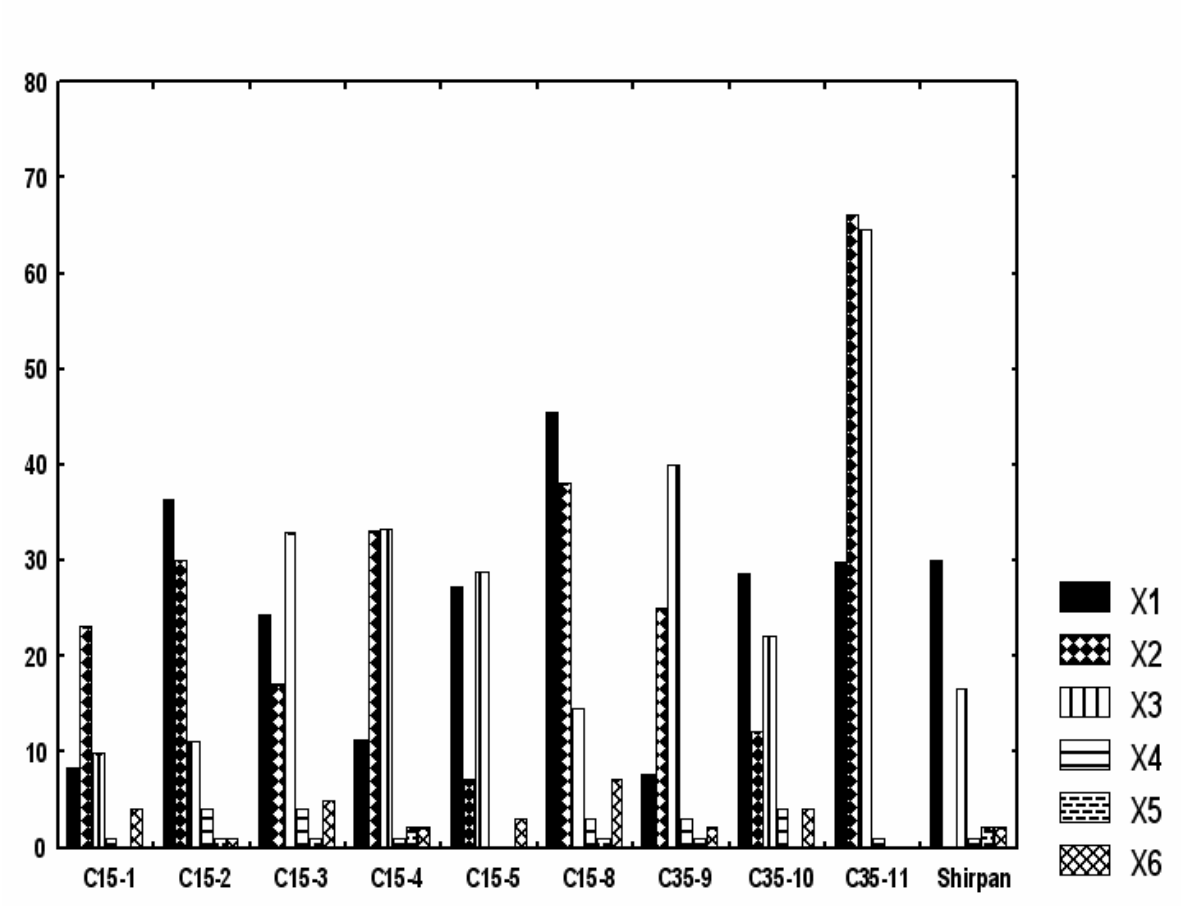


Figure 3. Percentage of meiotic cells showing abnormality. X1-X6 are meiotic characters 1-6 in Table 1.

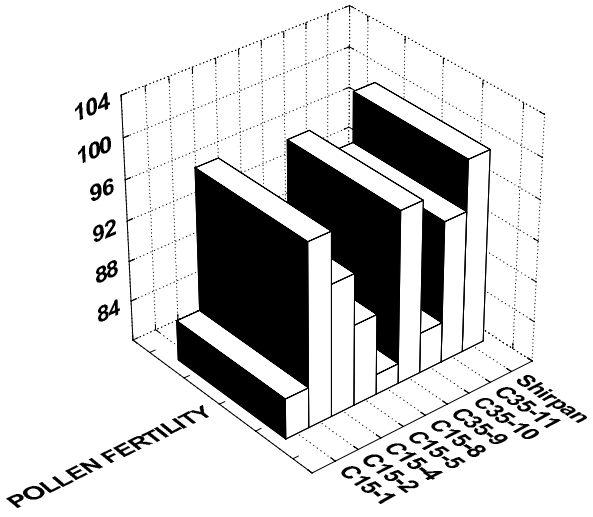


Figure 4. Pollen fertility (%) in cotton lines.

In factor two with about 27% of total variance, meiotic characters of abnormality in metaphase-I, chromosome stickiness, unequal segregation and cells with three quadrivalents possessed the highest correlation (>0.80). This factor separates C15-8 from the others. Therefore these are the most variable meiotic characters among the lines irradiated with 15 krads and Shirpan.

Cluster analysis and ordination of the cotton lines irradiated with 35 gray produced similar results (Figs. 7 and 8) in which two main clusters are formed. Shirpan alone stands in the first main cluster while, C35-9, C35-10 and C35-11 form the second main cluster. C35-11 shows more similarity to Shirpan compared to the other irradiated lines.

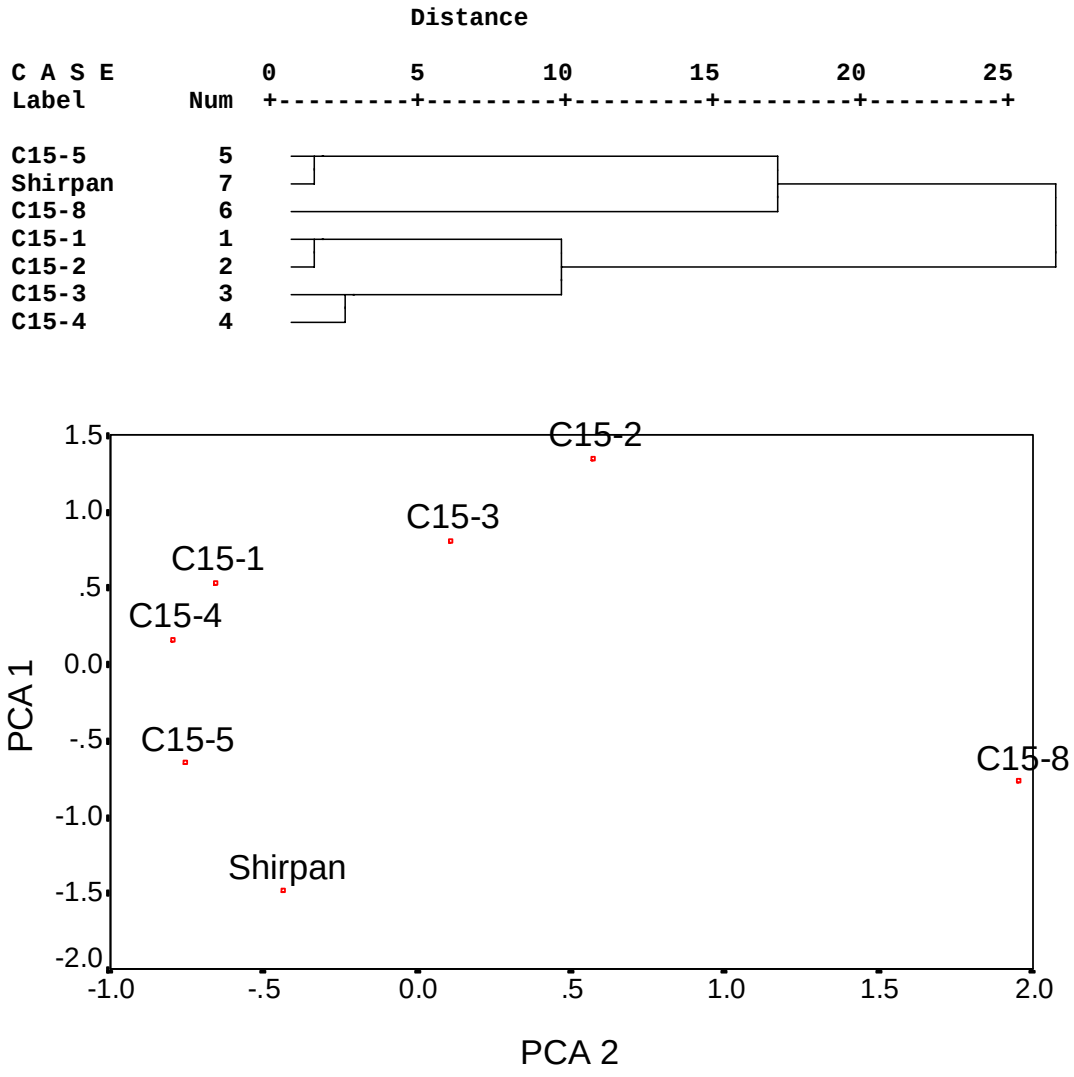
Factor analysis of meiotic data among cotton lines irradiated with 35 krads and Shirpan, revealed that the first two factors comprise about 76% of total variance. In the first factor which comprises about 45% of total

Table 4. Factor analysis of cytogenetical characters among cotton lines treated with 150 Gy gamma rays. Cytogenetical characters as in Table 1. The main body of the table indicates factor loadings of the characters

Character	Factor1	Factor2	Factor3	Factor4
1	-.26537	.81769	-.01226	-.41616
2	.46556	.55840	.17970	.54349
3	-.02239	-.38186	.78173	.13063
4	.57380	.68580	.19914	-.32543
5	-.24735	.03714	.70422	-.28823
6	-.18427	.60229	-.04659	.50577
7	.07782	.81206	-.42188	.32300
8	-.08658	.95346	-.02911	.13955
9	-.05653	.98484	.07621	-.09599
10	-.34438	-.00453	.63587	.00013
11	.42060	-.40063	.68207	.02260
12	-.97430	.16556	.12354	.01503
13	.96920	-.21634	-.08719	-.00698
14	.84490	.28783	-.40861	-.07861
15	-.03182	.46065	.82473	.23236
16	-.98515	.05426	.08742	.09889
17	-.59492	.37387	.66292	.23001
18	-.65170	-.19094	-.03115	-.57443
19	-.21567	.19219	-.09791	.92974
20	.42974	-.32820	.33928	.72105
21	.68757	-.10697	-.49699	.32879
22	-.33716	.86373	-.00711	.37363
23	.86620	-.30443	.11098	.29904
24	.73927	.39637	-.04167	.13793
25	-.97433	.16546	.12355	.01516
26	.97433	-.16546	-.12355	-.01516

Table 5. Factor analysis of cytogenetical characters among cotton lines treated with 350 Gy gamma rays. Cytogenetical characters as in Table 1. The main body of the table indicates factor loadings of the characters

Character	Factor1	Factor2	Factor3
1	.10079	-.98695	.12557
2	.94393	.02739	-.32902
3	.89668	.15645	-.41411
4	-.06271	.49445	.86694
5	-.85281	-.01435	-.52203
6	-.52075	.09841	.84802
7	.45766	.08765	.154678
8	.31554	-.27902	.90697
9	.48903	-.85065	-.19300
10	-.53163	-.83240	.15646
11	.99160	-.10117	-.08057
12	-.76895	-.63886	.02384
13	.74291	.66787	-.04522
14	.95278	.03539	.30159
15	-.69516	.63370	.33937
16	-.53439	-.77443	-.33865
17	-.98307	-.18048	-.03173
18	-.33716	.86373	-.00711
19	.02848	.84485	.53425
20	.08581	.95512	-.28352
21	.01051	-.15713	.98752
22	.82637	-.42160	.37333
23	-.02421	.99123	-.12989
24	.56159	.78764	.25346
25	-.71824	-.69545	.02385
26	.71824	.69545	.02385



Figures 5 & 6. Cluster analysis (UPGMA) and ordination of lines treated with 150 Gy gamma rays.

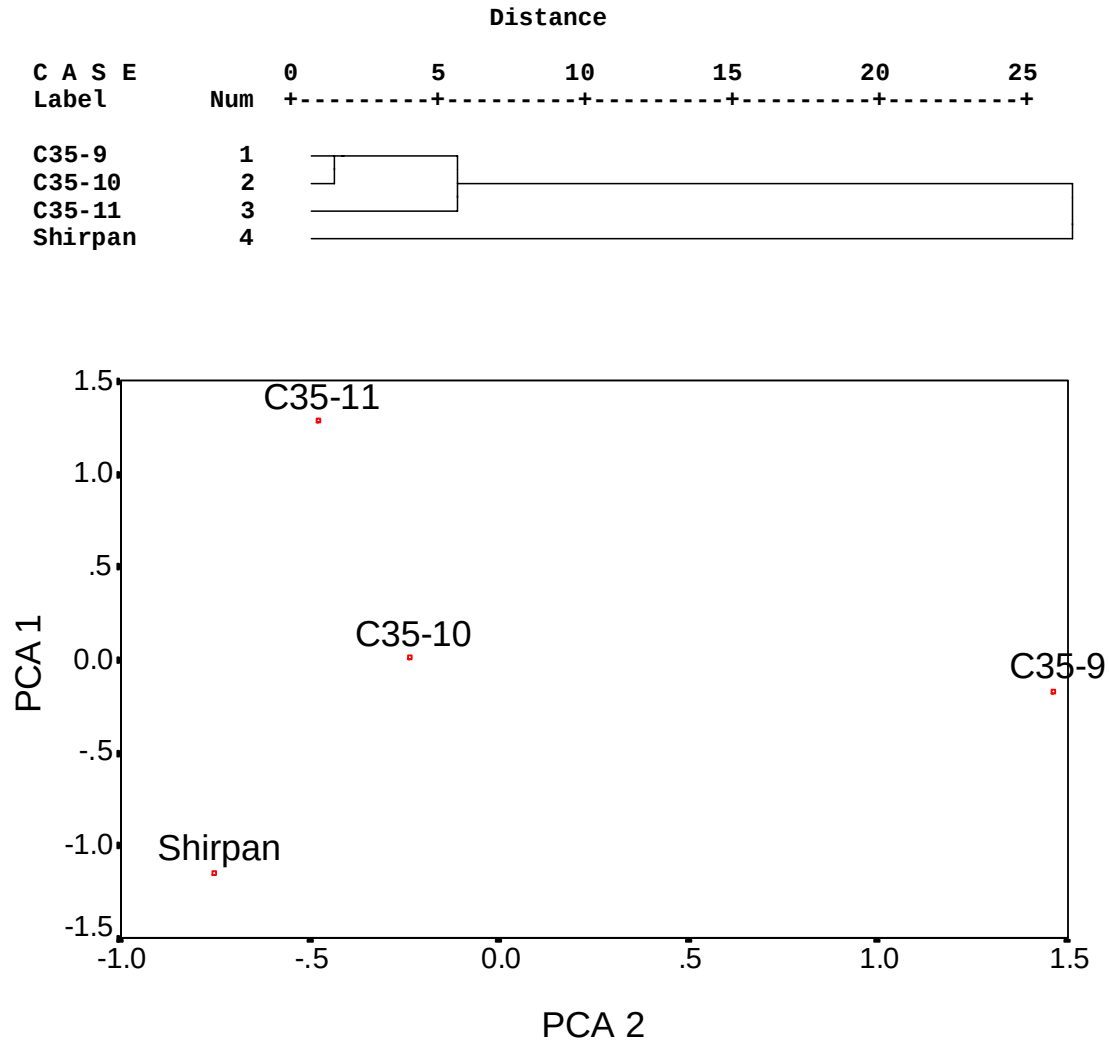
variance, meiotic characters like laggard chromosomes, abnormality in anaphase-I, bivalents with one chiasma and univalents, cells with telophase-I abnormality and cells with three quadrivalents (Table 5) possessed the highest correlation (>0.70).

In the second factor, which comprises about 30% of total variance, meiotic characters like adjacent and alternate quadrivalents as well as cells with one and 2 quadrivalents possessed the highest correlation (>0.70) (Table 4). These are the most variable meiotic characters among the lines treated with 350 Gy and the control line. The first factor separates Shirpan and C35-11 from irradiated lines, while factor 2, separates C35-9 from the other cotton lines.

Cluster analysis and ordination of all the cotton lines based on factor analysis is presented in Figures 9 and 10. The lines treated with 150 and 350 Gy irradiation are grouped together with no distinct separation. Therefore it seems that both doses produce some overlapping cytogenetic effects.

Diffuse Stage in Meiosis-I Prophase

The meiotic analysis of all 10 cotton lines studied including the control line (Shirpan), showed a deviant course of meiosis-I prophase sub-stages i.e. the occurrence of synezetic knot stage instead of leptotene and zygotene. In the early synezetic knot stage, thin

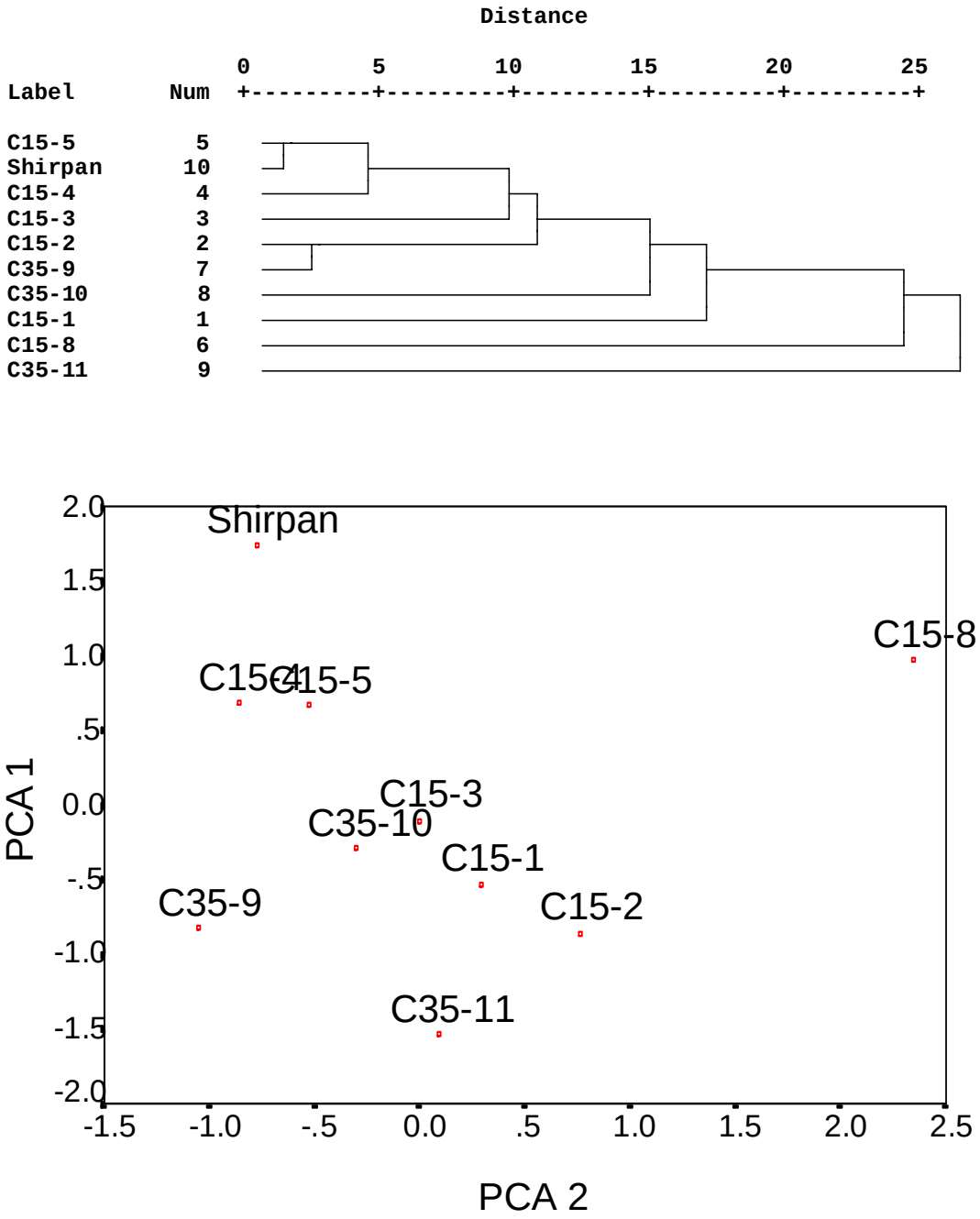


Figures 7 & 8. Cluster analysis and ordination of the lines treated with 350 Gy gamma rays.

chromatin strands surround the nucleolus till covering it totally. Later on paired chromosomes (now thick strands) unraveled from the knot, entering the pachytene stage (Fig. 1, 5). End to end attachment of chromosomes in pachytene is a feature reported in those taxa showing synezetic knot stage [8,17]. However despiralization of chromosomes occurred after pachytene, commencing diffuse stage in the irradiated lines only (Fig. 1, 5), and not in the control line (Shirpan). After diffuse, diplotene stage commences showing secondary contraction followed by diakinesis and metaphase stages.

Our earlier study of meiosis-I prophase in other *G. hirsutum* cultivars and their hybrids did not show the occurrence of diffuse stage [23]. The occurrence of diffuse stage has been reported in several plant species [25]. Diffuse may be of complete type in which the whole chromosomes decondense or it may be partial in which some parts of the genome show decondensation. The present study showed the occurrence of partial diffuse in cotton irradiated lines, irrespective of gamma rays dosage used.

Various reasons have been suggested for the occurrence of diffuse stage. These 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 [8,17]. It may be suggested that gamma irradiation is the reason for the occurrence of diffuse stage in irradiated lines studied.



Figures 9 & 10. Cluster analysis and ordination of the lines treated with 150 and 350 Gy gamma rays.

Cytomixis

Cytomixis and chromosome/chromatin migration was observed in some of the irradiated lines (Fig. 1, Table 2). The highest value of cells showing cytomixis occurred in C35-10 and C15-8 (6.00 and 5.00 respectively). Migration of chromatin material among the adjacent meiocytes occurs through cytoplasmic connections originated from the pre-existing systems of plasmodesmata formed within the anther tissues. The plasmodesmata become completely obstructed by the deposition of callose [7], 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 [7,18,19].

Chromatin/chromosome migration occurred in different directions from the early prophase to telophase-II stages in most of the mutant lines except C15-1, C15-4 and C35-9 and Shirpan. The chromatin materials migrated ranged from few small fragments to complete bivalents (Fig. 1, 1 & 2). Several metaphase cells possessed extra/ missing chromosomes showing aneuploid condition (Fig. 1, 3 & 4). Several deformed and infertile pollen grains were also observed possibly due to cytomixis (Fig. 1, 7).

Our earlier cytogenetical studies in *Gossypium herbaceum* and *G. hirsutum* cultivars as well as their hybrids did not show the occurrence of cytomixis or aneuploid gametes [19]. Therefore the gamma irradiation may be the reason for the occurrence of cytomixis in the present cotton lines.

Cytomixis leads usually to aneupoloidy and reduction in fertility of plants, therefore it is considered to be of less evolutionary significance [4,15]. However it may bring about new genetic variability by producing aneuploid gametes and new phenotypic characters as reported in other plants [15,18].

The significant difference observed in the frequency and type of quadrivalents (alternate versus adjacent) and other meiotic characteristics as well as morpho-agronomic characters (unpublished data) among gamma irradiated lines, may reflect partly their genomic differences as these plants were grown under uniform conditions in the experimental field. Such genomic variations if combined with other morpho-agronomic characters may be used in further breeding and selection in cotton.

The lines C15-1, C15-3, and C15-4 possess earliness, high yield and a proper length of lint (important agronomic characters in cotton) and may be selected for cultivation and propagation. C35-9 possesses all above characters except the yield. Line C15-2 possesses high yield and a good quantity of lint, but lacks earliness and quality of linter [12]. These cotton lines may be used in further hybridization and selection practices.

The cotton lines studied possessed a higher value of alternate quadrivalents compared to that of adjacent one (Fig. 2), but show a lower value of abnormality in metaphase-I and II. Therefore we may suggest that based on the present study, a higher number of alternate quadrivalents and lower value of metaphase abnormality in cotton lines may be related to a better performance of cotton lines.

Sine the present study considers only cytogenetic analysis of cotton lines treated with a limited range of Gamma ray doses, no conclusion may be drawn at present to identify the most suitable Gamma ray dose or doses for generating variability in tetraploid cotton. Further studies are required in this regard.

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