

CYTOLOGICAL STUDIES ON MUTANT LINES OF BREAD WHEAT

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ABSTRACT

In this investigation an attempt was made to study the effect of gamma radiations on mitotic activity, micronuclei formation and chromosomal aberrations, which are used as parameters for cytological instability in both mitotic and meiotic cell divisions. Another aim of this study was the determination of chromosome pairing associations and chiasma frequency using five wheat mutant lines and the original parent Sids1. The studied mutant lines and their original parent exhibited variation ranged from the highest score of mitotic index in line GWM4 to its lowest estimate only in the original parent. The results exhibited differences ranging from the highest score of abnormalities in the mutant line GWM2 to the lowest estimate in the mutant line GWM4 in mitotic divisions. However, the lines GWM1, GWM2 and GWM3 have higher percentage of cells containing either micronuclei or chromosomal aberrations in meiosis than mutant lines GWM4, GWM5 and the original parent Sids1. The cytogenetical results revealed that the highest percentage of pollen mother cells PMC's showing bivalents was obtained in the mutant lines GWM4 and GWM5. Thus, these lines could be considered the most stable in meiotic behavior. Non significant differences were found in mean chiasma frequency of bivalents, bivalents plus univalents and bivalents + multivalent associations between the original parent and mutant lines in all cases.

Key words: *Wheat – Cytology – Mutations – Chromosome aberrations*

INTRODUCTION

Wheat is one of the most important cereal crops in Egypt, either as a staple food grain for human or as a major source of straw fodder for animal feeding. Both bread wheat (*Triticum aestivum* L; $2n=6x=42$; A, B and D genomes *Turgidum* ssp. *Durum* L. $2n=4x=28$, A and B genomes) have multiple gene characterized by regions differing in gene density and distribution (Hohmann *et al* 1994 and Sandhu and Gill 2002). Irregular meiosis was observed in triticale controls (with laggards and univalents) but increased in frequency with increasing radiation dose in all species. (Major and Khanna 1988). In this respect, Arora *et al* (1989) reported that mitotic aberrations in the root tips, anaphase bridges and micronuclei at telophase were increasing by gamma irradiation. Gamma rays are known to influence plant growth and development by inducing cytological, genetical, biochemical, physiological and morphogenetic changes in cells and tissues (Rudolph 1971).

Recently investigations showed that *Ph1* gene affects the pairing of

homologous chromosomes (Wang 1990) and the dynamics of microtubules (Feldman 1993). The formation of multivalent may cause an irregular distribution of chromosomes during meiosis leading to formation of unbalanced gametes and subsequently increase the meiotic instability and reduce the fertility. Chiasma formation and chiasma frequency are known to influence chromosome and gene recombination. In general, a high chiasma frequency would indicate better pairing among the parental chromosomes involved (Jauhar and Joppa 1996).

The five glaucous mutant lines are characterized by a heavy epicuticular wax layer covering the stems, leaves and spikes and they have different morphological and agronomic characters, however, Sids1 is a non-glaucous spring wheat cultivar. Each of the developed mutant lines is characterized by a significant increase or decrease in at least one agronomic character as compared to other mutants and their parent (Al-Bakry 2007).

The present investigation was undertaken, using five mutant lines of bred wheat and their original parent Sids1, to obtain the following cytogenetical information.

1-Determining of mitotic cell divisions in mutant lines as an indication for the adaptability to the Egyptian conditions.

2-Determining frequency of micronuclei and chromosomal aberrations in mitotic and meiotic cell divisions as an indication of the cytological instability in mutant lines and original parent.

3-Evaluation of chromosome pairing associations.

4-Determining the chiasma frequency in the meiosis of the mutants.

MATERIALS AND METHODS

Five glaucous lines of bread wheat i.e. GWM1, GWM2, GWM3, GWM4, GWM5 in the 5th mutated generation (M5) and their original parent, Sids1, were used for cytogenetic analysis in the present study. The five glaucous wheat mutant lines were selected in M2 generation from a glaucous wheat mutant which resulted from irradiation of Sids1 cultivar with 30 Krad (Kilo Rad) of gamma rays in the first mutated generation (M1) in winter season, 2001/2002 (Al-Bakry 2004). In M3 and M4 generations, mutants were grown in spike to-row progenies. Irradiation treatment was achieved by a C0-60 (cobalt-60) Gamma irradiation Unit, Cyclotron Project, Nuclear Research Center, Atomic Energy Authority, Egypt. The five glaucous mutant lines are characterized by a heavy epicuticular wax layer covering the stems, leaves and spikes and they have different morphological and agronomic characters compared to each other and their parent (Al-Bakry 2007). The parent Sides1 is a non-glaucous spring wheat cultivar.

Cytogenetic analysis

For mitotic studies, grains from five mutant lines and their original parent were germinated on moist filter paper on Petri dishes at room temperature in a randomized complete block design experiment with three replications. Each replication comprised three dishes for each entry and each dish contained 15 grains. Actively growing root-tips were cut from the seedlings and fixed in Farmer solution. The aceto-carmine squash technique was used to stain the root-tip cells as described by Sayed-Ahmed (1985). Nine prepared slides were used for each mutant line and the original parent to determine the frequencies of mitotic index, micronuclei and chromosomal aberrations.

For studying meiosis, chromosome pairing associations and chiasma frequency, whole spikes of the mutant lines and original parent plants were collected at an appropriate stage, immediately fixed in a 3:1 alcohol/acetic acid solution for 24 hours. Then they were washed with distilled water several times before being stored in 70% ethanol. Squash preparations of pollen mother cells (PMC s) were made in aceto-carmine as described by (Fayed *et al* 1984). About 20 slides were prepared from 10 randomly selected plants for each mutant line or the original parent. The prepared slides were used to determine micronuclei and chromosomal aberrations in meiotic cells and the following cytogenetical characters were determined:

a- Chromosome pairing associations

The chromosome association aims to investigate the frequencies of various configurations, i. e. univalents, bivalents and multivalents at first metaphase stage. For this purpose, the frequencies of (PMC s) showing either only regular chromosome associations, i.e. bivalent (II) or irregular chromosome associations, i.e. bivalent (II) + univalent (I), bivalent (II) + univalent (I) + trivalent (III) and bivalent (II) + quadrivalent (IV) were scored.

b- Chiasma frequency

The total number of chiasma per cell were recorded in 72-101 well spread PMC s. This determination was based on the number of bound arms either in homologous chromosome pairing, i.e. in bivalents, or in homologous pairing, i. e. in trivalents and quadrivalents. The total number of chiasmata per line were pooled and their mean was used to represent chiasma frequency.

All data, arranged in a randomized complete blocks design and were statistically analyzed according to Snedecor and Cochran (1982).

RESULTS AND DISCUSSION

1- Mitotic activity

The data of mitotic activity, expressed by the mitotic index (MI), in the glaucous mutant lines of bread wheat i.e. GWM1, GWM2, GWM3, GWM4, GWM5 and their original parent, Sids1 are given in Table (1). This table showed that the variation in MI between the different glaucous mutant lines and control (Sids1) are observable. This variation ranged from the highest score of MI in GWM4 (16.77) to its lowest estimate (7.95) in the original parent (Sids1). Mitotic index in GWM2 and GWM3 was close to the highest score found in GWM4.

Table 1. Mitotic index (M. I.) and frequency of mitotic phases in mutant lines and original parent of bread wheat.

Lines	Total no. of studied cells	Total no. of divided cells	M.I.	Frequency of mitotic phase		
				Prophase	Metaphase	Anaphase
Sids1	3509	279	7.95	5.50	1.06	1.40
GWM1	3407	402	11.80	9.54	0.99	1.26
GWM2	3741	608	16.25	12.59	1.87	1.79
GWM3	3505	563	16.06	11.73	1.80	2.54
GWM4	3470	582	16.77	12.43	1.80	2.54
GWM5	3544	546	15.41	11.46	1.72	2.23

The high MI in GWM4, GWM2, GWM3 and GWM5, probably, indicated that these lines are more adapted to the Egyptian conditions than the other GWM1 and original parent, Sids 1 in which MI was sharply reduced. In this respect, Uppal and Maherchandani (1988) studied seeds of CV. C306, irradiated with 20 KR of gamma radiation at 370 R per minute and then soaked in water or solutions of 0-50 pp m gibberellic acid (GA3) for 16 hr. Cytological examination of root tips of the germinated seeds showed that increasing concentrations of GA3 reduced the frequency of chromosomal aberrations. It is suggested that GA3 may enhance peroxides activity which eliminates chromosome damaging peroxy radicals which are formed during gamma irradiation.

The decrease in MI could be attributed to the increase in length of interphase period (Dulout and Olivero 1984). The frequencies of interphase observed in the lines which showed low MI in the present study were obviously higher than of high MI, indicating the effect of gamma irradiation of prolonged interphase as suggested by the above authors.

2- Chromosomal aberrations and micronuclei in mitotic and meiotic cells

The data presented in Tables (2 and 3) showed that the percentage of cells containing either micronuclei or chromosomal aberrations in mitotic and meiotic divisions depended on the mutant lines and the original parent (Sids1). These differences ranged from the highest score of abnormalities in the line (GWM2) to the lowest estimate in the line (GWM4) in mitotic divisions. However, GWM1 and GWM3 exhibited higher frequencies of abnormalities than GWM5 and the original parent (Sids1). Table (3) also showed that most mutant lines (GWM1, GWM2 and GWM3) have higher percentage of cells containing either micronuclei or chromosomal aberrations in meiosis than the mutant lines GWM4, GWM5 and the original parent.

Table 2. Frequency of micronuclei and chromosomal aberrations in meristematic root tips of mutant lines and original parent of bread wheat.

lines	Total no. of studied cells	Total no. of divided cells	Percentage of		Percentage of micronuclei types		Percentage of types of chromosomal aberrations			
			Micronuclei	Chromosomal aberrations	compact	Non compact	fragments	stickiness	disulfate cells	aggards
Sids1	3509	279	7(0.20)	2(0.72)	(0.03)	(0.17)	-	-	(0.72)	-
GWM1	3407	402	8(0.25)	5(1.24)	(0.06)	(0.19)	-	(0.74)	(0.50)	-
GWM2	3741	608	9(0.24)	9(1.48)	(0.08)	(0.16)	-	(0.66)	-	(0.82)
GWM3	3505	563	13(0.37)	4(0.71)	(0.17)	(0.20)	(0.18)	-	(0.53)	-
GWM4	3470	582	-	-	-	-	-	-	-	-
GWM5	3544	546	3(0.08)	3(0.55)	-	(0.08)	(0.18)	-	(0.37)	-

() percentage.

Table 3. Frequency of micronuclei and chromosomal aberrations in PMC's of mutant lines and original parent of bread wheat.

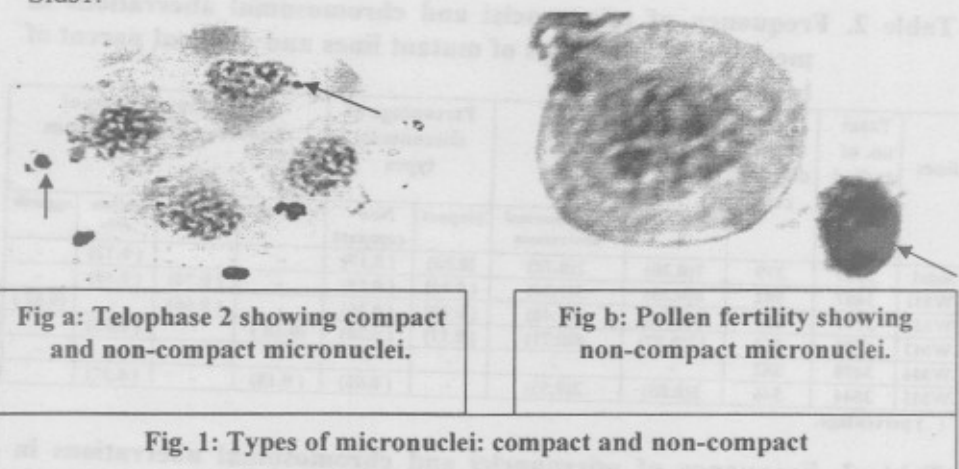
lines	Total no. of studied cells	Total no. of divided cells	Percentage of		Percentage of micronuclei types		Percentage of types of chromosomal aberrations				
			Micronuclei	chromosomal aberrations	compact	Non compact	fragments	stickiness	aggards	Unequal Distribution	Univalent
Sids1	1263	156	8(0.64)	8(5.13)	(0.24)	(0.40)	(0.64)	(1.92)	(1.28)	(1.28)	-
WM1	1011	238	8(0.79)	16(6.72)	(0.30)	(0.49)	(1.68)	(1.68)	(1.26)	(1.68)	(0.42)
WM2	1180	181	10(0.85)	12(6.63)	(0.34)	(0.51)	(1.10)	(2.21)	(1.10)	(1.64)	(0.55)
WM3	1064	175	7(0.66)	12(6.85)	(0.19)	(0.47)	(2.28)	(2.28)	(0.57)	(1.14)	(0.57)
WM4	1024	239	2(0.20)	-	-	(0.20)	-	-	-	-	-
WM5	1018	216	-	4(1.85)	-	-	(0.93)	(0.93)	-	-	-

() percentage.

The increased frequency of micronuclei and chromosomal aberrations in most mutant lines indicated the role of gamma irradiation for the occurring of micronuclei and chromosomal aberrations as reported by Uppal

and Maherchandani (1988). In this respect, Major and Khanna (1988) observed irregular meiosis in triticale controls (with laggards and univalents) but increased in frequency with increasing radiation dose in all species. Xie *et al* (1994) found chromosome aberrations in both un-irradiated and irradiated protoplasts, but irradiation apparently increased the frequency of chromosome aberrations.

The types of micronuclei compact and non-compact, previously described by Hesseman and Fayed (1982) in *Vicia faba* were also detected in mitosis and meiosis of the mutant lines. The existence of both types of micronuclei has not been previously reported in mutant lines. As Tables (2 and 3) showed, the frequency of non-compact micronuclei was in general, more than that of compact types in mutant lines and the original parent Sids1.



The various types of chromosomal aberrations were presented in Tables (2 and 3). The types observed in mitosis were stickiness, fragments, laggards and binucleate cells. In meiosis the observed types included stickiness, fragments, laggards and unequal distribution of chromosomes, i.e. anaphase II or telephase II showing only three nuclei (Figure 2). Binucleate cells and stickiness represented the most frequent types of chromosomal aberrations in mitosis and meiosis of the studied materials. The other kinds of aberrations were found in low frequencies.

3- Chromosome pairing association

The mean percentage of meiotic pairing associations in the mutant lines and the original parent are presented in Table (4). Although similar meiotic configurations in most mutant lines were noticed, the mean percentage of each pairing associations depended on which mutant line was considered. Considering bivalent configuration as an indication for regular

pairing, the data of Table (4) indicated that the percentage of PMC's showing only bivalent in mutant lines ranged from 65.55% in GWM2 to

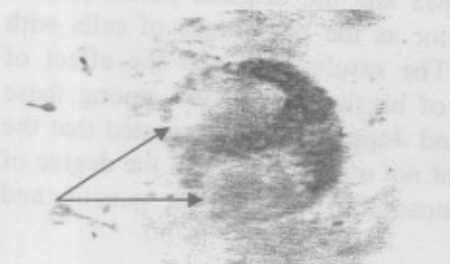


Fig a: Binucleate cell.



Fig b: Chromosomal bridge.

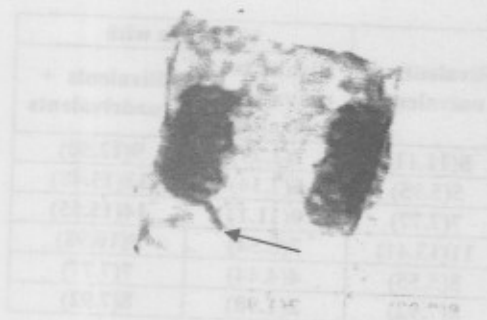


Fig c: Uncoiling chromosome.

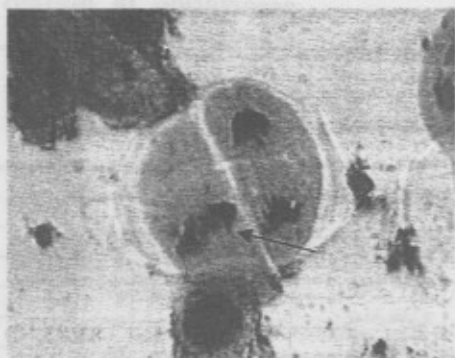


Fig d: Chromosome Stickiness.

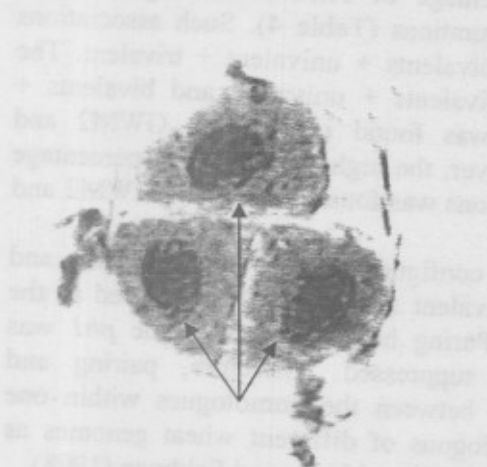


Fig e: Unequal distribution of only three nuclei.



Fig f: Univalent chromosome.

Fig 2. Types of chromosome aberration in mitotic and meiotic cell divisions.

bivalent (82.18 % and 73.61 %) and that with bivalent plus univalent 82.22% GWM4. The mutant line GWM5 and the original parent (Sids1) showed the most stable meiotic behavior as the percentage of cells with trivalent associations are considered. The results reflected the effect of gamma irradiation on the frequencies of bivalent formation among these mutant lines. In this respect, Jauhar and Joppa (1996) suggested that the amount of chromosome pairing in wheat not only depends on the degree of homology between the pairing chromosomes but also on genetic and environmental factors.

Table 4. Mean percentage of metaphase I chromosome pairing association in mutant lines and original parent of bread wheat.

Lines	No. of PMC's examined	Bivalents	Bivalents + univalents	% of cells with	
				Bivalents + univalents+ trivalents	Bivalents + quadrivalents
Sids1	72	53(73.61)	8(11.11)	2(2.77)	9(12.50)
GWM1	84	60(71.42)	5(5.95)	6(7.14)	13(15.48)
GWM2	90	59(65.55)	7(7.77)	10(11.11)	14(15.55)
GWM3	82	55(67.07)	11(13.41)	7(8.54)	9(10.98)
GWM4	90	72(82.22)	5(5.55)	4(4.44)	7(7.77)
GWM5	101	83(82.18)	8(7.92)	2(1.98)	8(7.92)

Interestingly, the mutant lines and the original parent (Sids1) exhibited considerable variations in the percentage of PMC's showing bivalents associated with other meiotic configurations (Table 4). Such associations included bivalents + univalent and bivalents + univalent + trivalent. The highest and lowest percentage of bivalents + univalent and bivalents + univalent + trivalent associations was found in GWM3, GWM2 and GWM4, GWM5, respectively. However, the highest and lowest percentage of bivalents + quadrivalents associations was found in GWM1, GWM2 and GWM4, respectively.

The formation of various configurations of homologous and homoeologous chromosomes, i.e. bivalent and multivalent, noticed in the present study, indicated that the "Pairing homoeologous" gene *ph1* was either absent or its effect was suppressed. Therefore, pairing and recombination took place not only between the homologues within one genome, but also between homoeologous of different wheat genomes as suggested by Sears (1976), Feldman (1993) and Vega and Feldman (1998).

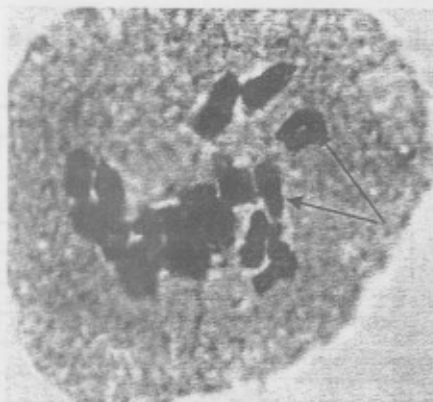


Fig a: Metaphase I showing only bivalents.

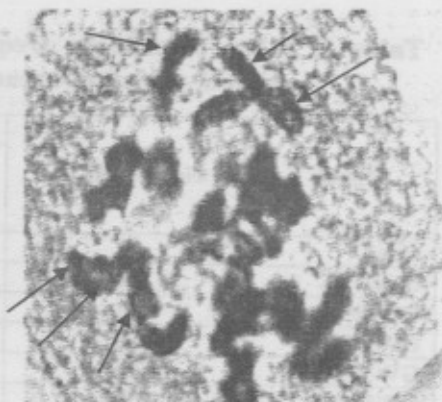


Fig b: 1 (arrow) univalent + 2 (arrows) trivalent + 3 (arrows) quadrivalent.

Fig 3: Chromosome pairing association, bivalent, univalent, trivalent, quadrivalent

4- Chiasma frequency

A- Chiasma frequency in bivalent configuration

The mean chiasma frequency of bivalents per PMC in the mutant lines and the original parent are given in Table (5). The mutant lines and the original parent varied in their chiasma frequency, showing different trends. The highest chiasma frequency per cell was exhibited by the original parent while the lowest one was displayed in the mutant line GWM3. Moreover, the mutant lines GWM1, GWM2, GWM4 and GWM5 showed similar chiasma frequencies. These results confirmed the opinion of Driscoll *et al* (1979) who stated that presynaptic association of homologous chromosomes in wheat is a prerequisite for the sequence of events that lead to chiasma formation. Moreover, Maguire (1995) reported that chiasma has been recognized as a major feature of meiosis, both visually and functionally, but its functional basis remains poorly understood. The present results, probably, suggested that the *ph1* gene was more operating in the lines showing high chiasma frequency. In this respect Jauhar *et al* (1991) found that chiasma frequency in the *ph1* bread wheat euploids was 7.5- 11.6 times higher than the *ph1* euploids.

Table 5. Mean of chiasma frequency in PMC's of mutant lines and original parent of bread wheat.

Lines	No. of PMC's	Mean of chiasma frequency in		
		Bivalents	Bivalents + univalent association	Bivalents + trivalent association
Sids1	72	42.47	39.00	44.00
GWM1	83	42.23	38.20	44.10
GWM2	90	42.25	38.00	43.54
GWM3	82	42.05	38.27	43.25
GWM4	90	42.32	38.80	43.64
GWM5	101	42.18	38.50	44.45

The differences in mean chiasma frequency in bivalent configuration noticed in the lines could be attributed to the differences in relative arm length and heterochromatin distribution as suggested by Ferrer *et al* (1984). Moreover, Miller and Reader (1987) concluded that chiasma frequency is specific for each bivalent.

b- Chiasma frequency in bivalent plus univalent and bivalent plus multivalent association

As expected the mean chiasma frequency per bivalent plus univalent association was reduced in all mutant lines and original parent (Table 5). The mutant lines GWM1, GWM2 and GWM3 exhibited the lowest mean chiasma frequency among the mutant lines and the original parent. In the other mutant lines and original parent, mean chiasma frequency of this association less affected the reduction in chiasma frequency of II+I association which gives a clear picture of univalent effects. As Table (5) indicated, the mean chiasma frequency of bivalent plus multivalent association ranged from 43.25 in mutant line GWM3 to 44.45 in mutant line GWM5. These results indicated that change in pairing configurations in the mutant lines occurred with change in chiasma frequency. In this respect, Reyes-Vaides and Stelly (1995) reported that, the frequencies of meiotic configuration in bread wheat depended on chiasma frequencies in segments defined by centromeres breakpoints and telomeres. Moreover, Jauhar and Joppa (1996) reported that by exercising proper selection, chiasma frequency and distribution may be changed and with that, the shape of meiotic configuration.

Results regarding the differences in mitotic index between the original parent and mutant lines are given in Table (6).

Table 6. Significance of the total number of mitotic index, micronuclei and chromosomal aberration in mitotic and meiotic division in the original parent and mutant lines.

Lines	Mitotic index	Micronuclei in mitotic divisions	Chromosomal aberrations in mitotic division	Micronuclei in meiotic division	Chromosomal aberration in meiotic division
Sids1	7.95 D	0.20 C	0.72 C	0.64 C	5.13 E
GWM1	11.80 C	0.25 B	1.24 B	0.79 B	6.72 A
GWM2	16.25 AB	0.24 B	1.48 A	0.85 A	6.63 A
GWM3	16.05 AB	0.37 A	0.71 C	0.66 C	6.85 A
GWM4	16.77 A	0.00 E	0.00 E	0.20 D	0.00 D
GWM5	15.41 B	0.08 D	0.55 D	0.00 E	1.85 C

Values within each column followed by the same letter are not statistically different at 5% level.

The data of this table showed that significant differences in mutant index between the original parent and mutant lines except mutant line GWM2 and GWM3, suggested that, differences in mitotic index could be attributed to the effects of gamma radiations on the studied strains. From Table (6), significant differences could be seen in number of cells containing either micronuclei or chromosomal aberrations between the original parent and mutant lines in mitotic and meiosis in many cases. From (Table 7), it can be seen that there were significant differences in number of PMC's showing the different types of chromosome pairing association between the studied strains in many cases. From Table (8), it can be seen that there were no-significance in mean chiasma frequency of bivalents, bivalents plus univalents and bivalents + multivalent associations between the original parent and the mutant lines in all cases.

Table 7. Significance of the total number of bivalents, bivalents plus univalent, bivalents + univalents + trivalents and bivalents + quadrivalents association in original parent and mutant lines.

Lines	Bivalent	Bivalent + univalent	Bivalent + univalent + trivalent	Bivalent + quadrivalents
Sids1	73.61 B	11.11 B	2.77 E	12.50 D
GWM1	71.42 C	5.95 D	7.14 C	15.48 A
GWM2	65.55 E	7.77 C	11.11 A	15.55 A
GWM3	67.07 D	13.41 A	8.54 B	10.98 C
GWM4	82.22 A	5.55 D	4.44 D	7.77 D
GWM5	82.18 A	7.92 C	1.98 E	8.91 D

Values within each column followed by the same letter are not statistically different at 5% level

Table 8. Significance of chiasma frequency in bivalents, bivalents + univalents and bivalents + multivalents in the original parent and mutant lines.

Lines	Mean of chiasm frequency in		
	Bivalents	Bivalents + univalent association	Bivalents + multivalent association
Sids1	42.47 A	39.88A	44.00A
GWM1	42.23 A	38.20A	44.10A
GWM2	42.25 A	38. 00A	43.54A
GWM3	42.05 A	38. 27A	43.25A
GWM4	42.32 A	38. 80A	43.64A
GWM5	42.18 A	38. 50A	44.45A

Values within each column followed by the same letter are not statistically different at 5% level

In the light of the results of mitotic activity in the glaucous mutant lines GWM4, GWM2, GAM3 and GWM5, probably, indicated that these lines are more adapted to the Egyptian conditions. From the cytological point of view, mutant lines GWM4 and GWM5 proved to be the best lines because they showed lower chromosomal aberrations than the other lines. According to the results of chromosome associations and mean chiasma frequency, chromosome pairing was predominantly bivalents with varied occurrence of multivalents. The glaucous mutant lines GWM4 and GWM5 showed the most stable meiotic behavior as for as the percentage of cells with bivalent configuration and its chiasma frequency are considered. Therefore, the breeder must considered, not only, the parental materials for hybridization, but also the behavior of chromosome pairing and chiasma frequency to establish a basis for the prediction of the cytogenetically stable genotypes. Moreover, chromosome pairing and recombination result in the release of variation upon which selection is practiced in plant breeding.

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دراسات سيتولوجية على سلالات طفرية من قمح الخبز

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أجري هذا البحث بهدف دراسة تأثير شدة جلا على نشاط الميتوزي وكذلك تكوين النسوي الصغيرة والتغيرات الكروموسومية كأحد الأدلة على عدم الثبات السيتولوجي وذلك في كل من الإنقسام الميتوزي والميوزي للطوافر الناتجة من المعاملة بثلاثة جلا. وكان الهدف الآخر من البحث هو دراسة الإرتباطات الكروموسومية وتكرار تكوين الكيتزما مستخدماً (5) سلالات طفرة من القمح في الجيل الخامس بجانب الأب الذي نشأت منه (الصنف سمس 1). أظهرت الدراسة بالنسبة للإنقسام الميتوزي وجود تباين واضح حيث أظهرت السلالة الطافرة (4) أعلى معدل في الإنقسام الميتوزي في حين أظهر الأب الذي نشأت منه (الصنف سمس 1) أقل معدل في الإنقسام الميتوزي كما أظهرت للدراسة أن السلالة الطافرة (2) أعطت أعلى معدل بالنسبة للإحرفات الكروموسومية في حين أظهرت السلالة (4) أقل معدل من الإحرفات الكروموسومية داخل الإنقسام الميتوزي بينما أظهرت السلالات الطافرة (1، 2، 3) معدلات عالية من التغيرات الكروموسومية مقارنة بالسلالات الطافرة (4، 5) والأب الذي نشأت منه (الصنف سمس 1) داخل الإنقسام الميوزي. أظهرت الدراسات السيتولوجية أعلى معدل لتكوين الإقتران الكروموسومي التتالي في السلالات الطافرة (4، 5) مما يوضح أنها تمتلك ثبات ميوزي عالي كما أوضحت النتائج أنه لا توجد فروق معنوية في معدلات تكرارات الكيتزما سواء في الإقتران التتالي أو الإقتران التتالي الكروموسومي + الكروموسومات القريبة أو الإقتران التتالي الكروموسومي + الإرتباطات الكروموسومية العديدة في كل الحالات. وبذلك تظهر النتائج أن التربية بالطفرات كان لها تأثير فعال في استحداث تباينات نافعة أتت إلى انتخاب سلالات متوازنة سيتولوجيا وخصبة يمكن الاستفادة الكاملة منها في برامج تحسين القمح. ويجب على المربي أن يفكر ليس فقط في الآباء التي تكفل في برنامج التربية ولكن أيضا في سلوك الاتحادات الناتجة من الإقتران الكروموسومي وتكرار الكيتزما لتساعد على بناء قاعدة للحصول على تركيب وراثية ثابتة من الناحية السيتولوجية.

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