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Research Article
Cytological Effect of Gamma Radiation on Selected Mutants of Wheat *Triticum aestivum* L. in M3 Generation

Hussah I. Algwaiz

Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, P.O. Box 84428S, Riyadh, Saudi Arabia

Abstract

Background and Objective: Wheat (*Triticum aestivum* L.) offers some unique opportunities for the induction and exploitation of agronomic value. The use of gamma radiation has been proven to be an effective method to induce genetic variation in crops. We aimed to determine genetically stable mutants of wheat which could be utilized for breeding purposes. **Materials and Methods:** We did a cytological investigation of induced mutant's behavior and chiasma frequency. Selected mutant types induced in dry and soaked seeds were treated with different doses of gamma rays. Each treated sample and control were subjected to cytological examination of the fixed pollen mother cells in various meiotic stages. **Results:** The percentage of the total abnormal cells significantly increased in one mutant and significantly decreased in the other mutant. The percentage of total abnormal cells did not diminish from the first to the second meiotic division. The types of meiotic anomalies found included laggards (56.51%), univalent (9.43%), stickiness (45.45%) and bridges (19.32%). There were genotypic differences in the frequency of occurrence of multivalent (trivalent and quadrivalents). A marked reduction in the number of rod and ring bivalent/cell in some genotypes were noticed. The frequency of chiasmata per pollen mother cell was reduced subsequently. Depression index of mutants was negative compared with controls or treatments except for a few genotypes. **Conclusion:** Selected mutants of wheat tend to be cytologically stable and can therefore, be utilized for breeding purposes.

Key words: *Triticum estivum* L, gamma radiation, third mutation, genetic variation, wheat

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Corresponding Author: Hussah I. Algwaiz, Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, P.O. Box 84428S, Riyadh, Saudi Arabia

Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Wheat (*Triticum aestivum* L.) offers some unique opportunities for the induction and exploitation of agronomic value. The use of gamma radiation has proved to be an effective method to induce useful mutation in several crops including wheat, red pepper, okra and maize[1-5].

Induction of genetic variation through gamma irradiation induced higher genetic variation of up to 4 times in Bambara groundnut[6]. The use of ionizing radiation (including x-rays, gamma rays, neutrons and chemical mutagens) has been used to improve major crops including wheat, barley, cotton, peanuts and beans[7]. It is considered the only accurate and efficient judgment for the achieved genetic stability and regeneration in the progenesis of selected mutants[8,9]. Some mutagens directly alter specific chromosomal proteins and these chromosomal aberrations occur during meiotic division[10]. Chromosomal aberrations including laggards, c-mitosis, multipolar chromosomes with or without spindles, stickiness, premature bivalent, tripolar cells, fragments and bridges, dysjunction and micronuclei occurred following irradiation of *T. aestivum* L.[11-13].

However, despite the well-known effect of gamma rays on plant growth and development by inducing morphological, cytological and physiological changes in the cells and tissues, there are few studies that investigated the genetic stability to yield a better agronomic value of wheat. Therefore, we aimed to determine genetically stable mutants which could be utilized for breeding purposes.

MATERIALS AND METHODS

Study setting and date of study: This study was conducted at the Department of Biology laboratory of Princess Noura bint Abdulrahman University in Riyadh, Saudi Arabia between May and December, 2018 (7 months).

Sample collection and preparation: Samples were collected from the markets in Riyadh, Saudi Arabia. In this study, samples were divided into 2, dry and soaked seeds and were treated with different doses of gamma rays. Each treated sample and control were subjected to cytological examination of the fixed pollen mother cells in various meiotic stages.

Irradiation of samples: Dry and soaked seeds of 3 lines of bread wheat L (5-130), (17-41-90) and (15-3-83) named L1, L2 and L3 were irradiated with 500, 5,000 and 10,000 rad of gamma rays. Treated and untreated seeds were grown in a completely randomized block design with 4 replications in wire cage houses. Selected mutants were carried out using the induction mutations in plant breeding suggested by Gottchalk and Wolff[14]. Table 1 shows the mutant strains and the controls used in the study.

Cytological examination: For the cytological study in M3, immature spikes from mutants, their treatments and control were fixed in Camoy's mixture (6 parts absolute ethanol: 3 parts glacial acetic acid and 1 part chloroform) for 24 h at room temperature. Fixed spikes were washed with 70% ethanol twice and kept in the refrigerator until used. Anthers of suitable size were smeared in acetocarmine stain.

Table 1: Mutant strains against their respective control

Line	Mutant No.	Treatment in Krad	Origin	Heading date	Kernel weight	Grain yield
1	-	Control	Dry	84.30	15.60	450.50
1	12	10	Dry	87.80**	21.90*	556.70
1	-	Control	Soaked	83.60	18.20	509.03
1	7	5	Soaked	70.50**	16.70	443.00
1	19	5	Soaked	96.80**	15.70	605.90
2	-	Control	Dry	68.90	22.40	457.90
2	2	5	Dry	68.30	24.80	642.90*
2	-	Control	Soaked	68.90	22.90	470.70
2	17	5	Soaked	69.90	24.50	602.90*
3	-	Control	Dry	80.20	13.80	412.80
3	6	0.5	Dry	76.80**	21.03**	805.80**
3	8	5	Dry	69.90**	17.40	569.90*
3	-	Control	Soaked	76.90	17.96	554.80
3	3	10	Soaked	75.60	21.70	833.30**
LSD:	0.05			2.01	4.74	126.30
	0.01			2.72	6.39	170.63

**Highly significant, *Significant

Analysis of samples: The types and frequencies of meiotic irregularities were recorded at metaphase I (MI), anaphase I (AI), metaphase II (MII) and anaphase II (AII). Chiasma frequencies were determined from MI. The percentage of reduction in chiasma frequency was measured as depression index using the equation:

$$\text{Depression index} = \frac{\text{A-B}}{\text{A}} \times 100$$

where, A is the number of chiasma in control plants and B is the number of chiasma in the mutant or treated plants.

Statistical analysis: Data was analyzed using the Statistical Package for Social Sciences (SPSS) version 23.0 (SPSS Inc, Armonk, New York, USA). Results were expressed as numbers and percentages for categorical variables and as mean and standard deviation for continuous variables. Analysis of variance (ANOVA) was done and the least significant difference (LSD) was taken for comparison between genetic materials. One-way analysis of variation (ANOVA) was used to test the significant difference between 2 groups. A $p<0.05$ was considered statistically significant.

RESULTS

The cytological data for the different genetic materials (genotypes) examined are summarized in Table 2-5. Table 2 shows that the genotypes exhibited significant differences at meiotic stages.

The number of pollen mother cells (PMC's) observed and the number of abnormal cells in each stage of meiotic division for genotypes is shown in Table 3. The percentage of abnormal cells showed a significant decrease in some meiotic stages for all mutants when compared with the control or treatments except mutant-19 which showed a significant increase for MI and AII and mutant-2 in MI. The percentage of abnormal PMC's did not decrease from MI-MII.

Abnormal cells: The percentage of total abnormal cells, types of meiotic anomalies as laggards, univalent, stickiness and bridges are shown in Table 4. The percentage of total abnormal cells showed significant decrease and increase for mutant-12 and 19, respectively. Another variable proportion at MI of cells was univalent (1.49-9.43%) for control (L2 cs) and mutant-19, respectively. The univalent accumulated in the equatorial region during MI and gave rise to lagging chromosome at AI in PMC's. Sticky metaphases were observed in the genetic materials. The lowest percentage was 28.31% for mutant-19. At ana-telophase stages, bridges were observed. The variable proportion percentage ranged from 5.04-19.32%.

Chiasma frequencies: Table 5 shows the chiasma frequencies in wheat which showed marked reduction in the mean number of rod and ring bivalent per cell for mutant-7 and mutant-19 compared to the control and treatments. The maximum extent of reduction in the mean number of rod and ring bivalent/cell was 7.66±0.57 and 7.44±0.47 for mutant-7 and 19, respectively. Multivalents ranged from trivalents to quadrivalents. There were genotypic differences in the frequency of occurrence of multivalents.

Depression index: Depression index was negative when mutants were compared with the control except for mutants-3, 8, 17 and 19. Negative values were obtained when mutants were compared with their treated counterparts except for mutant-19. The frequency of chromosomal aberrations in the genotype were as near as the control except for one mutant. On the contrary, chiasma frequency increased as the control for the mutants. These results indicated that selected mutants tend to be cytologically stable.

DISCUSSION

This study showed that the genotypes exhibited significant differences at meiotic stages. Apparently, genetic differences within this population may have contributed to the differences in the stages among genotypes. Furthermore, the significant decrease in the percentage of abnormal cells in some meiotic stages for all mutants can be attributed to either no recovery or no elimination of abnormal PMC's during the course of meiotic division. However, this finding is in contrast to that of Kalinka *et al.*[15] where they reported that elimination of PMC's occur before and after meiosis as well as in each stage of meiotic division. They further suggested

Table 2: Mean squares for the percentage of abnormal cells in different meiotic stages of wheat (control, treatments and their mutants) in M3 generation

Variables	df	Metaphase I (MI)	Anaphase I (AI)	Metaphase II (MII)	Anaphase II (AII)	Total of abnormal cells (%)
Rep.	3	25.65	33.15	35.75	22.73	8.31
Genotypes	20	34.64**	102.31*	109.86**	53.31**	24.63*
Error	60	8.48	46.64	14.06	21.45	10.23

**Highly significant, *Significant

Table 3: Number and percentage of abnormal meiotic cells of wheat (control, treatments and their mutants) in M3 generation

		Metaphase I (MI)			Anaphase I (AI)				Metaphase II (MII)			Anaphase II (AII)			
Genotypes	Pedigree	No. of exam cells	No. of abn. cells	Percentage	No. of exam cells	No. of abn. cells	Percentage	Mean percentage of MI and AI	No. of exam cells	No. of abn. cells	Percentage	No. of exam cells	No. of abn. cells	Percentage	Mean percentage of MII and AII
L1	C D	223	29	13.00	88	14	15.90	14.45	60	9	15.00	54	9	16.66	15.85
L1	10 kr D	215	31	14.42	88	12	13.64	14.07	60	5	8.33	54	7	12.96	10.65
Mut-12	10 kr D	247	24	9.72^{+}	109	9	8.26	8.99	89	5	5.62**	77	6	7.79**	6.71
L1	C S	237	23	9.70	123	18	14.63	16.16	70	11	15.71	51	6	11.76	13.74
L1	5 kr S	214	33	15.42	143	14	9.79	12.61	75	10	13.33	92	13	14.13	13.73
Mut-7	5 kr S	298	34	11.41	176	8	4.55*	7.98	59	6	10.17*	60	6	10.00	10.09
Mut-19	5 kr S	280	49	17.50**	130	31	23.85	20.58	71	12	16.90	75	14	18.66*	17.78
L2	C D	327	22	6.72	69	13	18.80	12.76	53	12	22.60	50	10	20.00	21.30
L2	5 kr D	235	38	16.17	123	16	13.01	14.59	71	10	14.08	75	12	16.00	15.04
Mut-2	5 kr D	269	30	11.50^{+}	58	7	12.07	11.61	76	6	7.89**	47	6	12.50*	10.19
L2	C S	264	44	16.16	99	13	13.13	14.90	67	4	5.97	55	6	10.90	8.40
L2	5 kr S	297	34	11.40	69	16	23.20	17.30	53	13	24.50	62	12	19.35	21.91
Mut-17	5 kr S	228	25	10.96*	70	8	11.43^{+}	11.19	56	6	10.71^{++}	61	5	8.19^{++}	9.45
L3	C D	188	35	18.60	80	11	13.75	16.18	101	5	4.95	35	5	14.28	9.62
L3	0.5 kr D	272	25	9.19	71	7	9.86	9.50	89	13	14.60	86	11	12.79	13.69
Mut-6	0.5 kr D	202	26	12.87**	71	8	11.27	12.08	42	3	7.14^{++}	97	10	10.31	8.73
L3	5 kr D	229	34	14.85	99	12	12.12	13.49	70	10	14.28	55	8	14.55	14.42
Mut-8	5 kr D	140	21	15.00	68	4	5.88	10.44	52	1	1.92^{++}	53	6	11.32	6.62
L3	C S	247	26	10.52	91	12	13.19	11.85	111	10	9.01	57	7	12.28	10.65
L3	10 kr S	171	26	15.20	69	10	14.49	14.80	54	5	9.25	54	7	12.90	11.08
Mut-3	10 kr S	182	25	13.70	71	5	7.04	10.37	71	5	7.04	46	4	8.69	7.87
LSD:	0.05			4.12			9.65				5.30				
	0.01			5.48			12.86				7.03				

*,**Significant between mutant with control, $^{+,++}$ Highly significant between mutant with treatment, L: Line, Mut: Mutant, D: Dry seeds, S: Soaked seeds, Abn: Abnormal, kr: Kilorad

Table 4: Percentage of the occurring abnormalities on meiosis of wheat (control, treatment and their mutants) in M3 generation

			Abnormal PMC's		Percentage of different abnormalities relative to the total number of abnormal cells			
Genotypes	Pedigree	No. of PMC's exam	N	Percentage	Laggard	Univalent	Sticky	Bridge
L (1)	C D	425	61	14.35	47.54	3.27	36.06	13.13
L (1)	10 kr D	477	55	11.53	39.99	3.64	41.82	14.55
Mut-12	10 kr D	522	44	8.43*	45.45	6.82	34.09	13.64
L (1)	C S	481	58	12.05	39.66	6.89	37.92	15.53
L (1)	5 kr S	524	70	13.36	50.00	2.85	34.30	12.85
Mut-7	5 kr S	593	54	9.11	48.15	3.70	37.04	11.11
Mut-19	5 kr S	549	106	19.03**	45.28	9.43	28.31	16.98
L (2)	C D	499	57	11.42	38.59	5.26	36.83	19.32
L (2)	5 kr D	504	76	15.08	40.78	2.63	39.46	17.13
Mut-2	5 kr D	450	49	10.89	53.06	4.08	34.69	8.16
L (2)	C S	485	67	13.81	35.82	1.49	44.78	17.91
L (2)	5 kr S	481	75	15.59	46.66	2.66	36.02	14.66
Mut-17	5 kr S	415	44	10.60	36.36	4.55	45.45	13.64
L (3)	C D	404	56	13.86	46.42	5.36	35.71	12.51
L (3)	0.5 kr D	518	55	10.61	40.00	3.64	43.63	12.73
Mut-6	0.5 kr D	412	47	11.41	51.06	2.13	34.04	12.77
L (3)	5 kr D	453	61	13.47	40.98	3.28	40.98	14.76
Mut-8	5 kr D	313	32	10.22	40.62	3.13	43.75	12.50
L (3)	C S	506	55	10.87	50.91	3.64	30.90	14.55
L (3)	10 kr S	348	48	13.79	43.75	4.17	39.58	12.50
Mut-3	10 kr S	370	39	10.54	56.51	5.12	33.33	5.04
LSD:	0.05			4.52				
	0.01			6.02				

*Significant, **Highly significant, kr: Kilorad D: Dry seeds, S: Soaked seeds, PMC: Pollen mother cells, L: Line, Mut: Mutant, N: Number

that the phenomenon of direct chromosome/chromatin elimination from PMC's leads to irregular meiosis and disturbances in the meiotic process and nucleoli are the first ones to be eliminated.

The significant decrease in the percentage of total abnormal cells can be attributed to homozygosity, genetic stability and recovery from irradiation damage. Genetic materials have exerted some effect on spindle formation or action as manifested by the presence of variable proportions of cells with laggard (35.8-56.5%) similar to findings by Kumar and Rai[16,17]. Chromosomal anomalies including stickiness, univalent, multivalents at metaphase and bridges, laggards and polyads are found at anaphase and telophase stages[18]. Univalents may result from the failure of chromosomes to pair at zygotene (asynapsis) or from the disjunction of homologous chromosomes at diplotene (desynapsis) because chiasma formation did not occur. The functional gametes, formed from such abnormal cells may result in an aneuploidy progeny and may induce significant changes in multiple phenotypic traits including cytosine DNA methylation patterns[19]. Chromatin stickiness may be caused by some changes in the surface properties of the chromosomes which caused them to adhere to each other. Metaphases with sticky chromosomes loses their normal appearance and they appear as agglomerated chromosomes due to effect of pollutants and chemical compounds where complexes are formed with the phosphate groups in DNA, condensation of the DNA or formation of chromatid cross links[20]. The formation of bridges may be due to the broken ends containing centromere from 2 different chromosomes that unite to form dicentric chromosomes or chromosomes are clumped together due to stickiness and are unable to separate completely at anaphase, or centromere inactivation resulting in dicentric chromosomes[21,22]. The marked reduction in the mean number of rod and ring bivalent/cell for mutant-7 and 19 and the presence of multivalents were also found in this study. Multivalents ranged from trivalents to quadrivalents. There were gentotypic differences in the frequency of occurrence of multivalents.

The differences in chiasma frequency genotypes indicated that the formation of chiasma is controlled by polygenes and it has a profound effect on the distribution of the various chromosome configurations at meiosis. Chiasmata that holds homologous chromosomes together prevent premature disjunction and if separated can result in laggard chromosomes[23]. This may be due to the homozygosity, genetic stability and general recovery from the irradiated damage in M3 generation. On the contrary, chiasma frequency increased as the control for the mutants.

Table 5: Chiasma frequencies in wheat (control, treatments and their mutants) in M3 generation

			Bivalent							Depression index	
Genotypes	Pedigree	No. of exam cells	Ring/cell	Rod/cell	Univalent/cell	Trivalent/cell	Quadrivalent/cell	Chiasma/cell	Chiasma/bivalent	C/mut	Treat/mut
L1	C D	51	10.96±0.64	9.74±0.65	0.098	0.137	0.059	30.53±0.66	1.45±0.03		
L1	10 kr D	59	11.75±0.54	9.08±0.54	0.051	0.051	0.068	29.91±0.55	1.43±0.026		
Mut-12	10 kr D	55	8.42±0.59	12.34±0.63	0.109	0.109	0.022	33.11±0.68	1.58±0.032	-8.45	-10.69
L1	C S	64	10.78±0.55	9.68±0.52	0.093	0.188	0.259	30.53±0.52	1.45±0.002		
L1	5 kr S	49	10.27±0.69	10.29±0.64	0.122	0.204	0.114	31.61±0.72	1.51±0.03		
Mut-7	5 kr S	42	7.66±0.57	13.10±0.58	0.119	0.119	0.002	33.86±0.64	1.61±0.03	-10.91	-7.11
Mut-19	5 kr S	46	13.04±0.49	7.44±0.47	0.252	0.152	0.086	27.92±0.65	1.33±0.02	8.54	11.67
L2	C D	55	10.15±0.44	10.52±0.45	0.109	0.109	0.112	31.41±0.43	1.49±0.021		
L2	5 kr D	61	11.87±0.49	8.75±0.45	0.049	0.148	0.183	29.96±0.48	1.43±0.023		
Mut-2	5 kr D	55	9.82±0.62	10.99±0.62	0.036	0.109	0.045	31.82±0.62	1.51±0.029	-1.31	-6.21
L2	C S	49	10.42±0.53	10.46±0.53	0.041	0.022	0.057	31.36±0.53	1.48±0.025		
L2	5 kr S	48	11.75±0.46	8.85±0.43	0.083	0.208	0.109	29.71±0.43	1.41±0.021		
Mut-17	5 kr S	48	10.54±0.53	10.07±0.55	0.083	0.208	0.099	30.68±0.55	1.46±0.026	2.16	-3.26
L3	C D	52	10.54±0.52	10.08±0.51	0.115	0.154	0.111	31.11±0.49	1.48±0.023		
L3	0.5 kr D	44	11.38±0.42	9.25±0.43	0.068	0.068	0.234	30.45±0.42	1.45±0.020		
Mut-6	0.5 kr D	43	8.35±0.38	12.47±0.38	0.064	0.069	0.042	33.47±0.35	1.59±0.018	-7.59	-9.92
L3	5 kr D	46	11.15±0.55	9.35±0.56	0.217	0.195	0.088	30.52±0.55	1.45±0.03		
Mut-8	5 kr D	41	10.97±0.44	9.76±0.44	0.122	0.073	0.075	30.83±0.47	1.46±0.02	0.900	-1.01
L3	C S	45	10.11±0.60	10.66±0.61	0.110	0.022	0.098	31.76±0.61	1.51±0.029		
L3	10 kr S	50	10.78±0.58	9.92±0.58	0.120	0.080	0.010	30.86±0.58	1.48±0.028		
Mut-3	10 kr S	48	10.56±0.66	10.15±0.66	0.080	0.160	0.050	31.42±0.65	1.49±0.031	1.07	-1.81

L: Line, Mut: Mutant, D: Dry seeds, S: Soaked seeds, Treat: Treatment, C: Control, kr: Kilorad

CONCLUSION

Meiotic anomalies including laggards, univalent, stickiness and bridges with irradiation were found with gamma radiation. Genotypic differences occur as trivalents and quadrivalents. There were also marked reduction in the number of rod and ring bivalent/cell and also with the frequency of chiasmata/pollen mother cell. Depression index of mutants was negative in most of the genotypes. Selected mutants of wheat tend to be cytologically stable and can therefore be utilized for breeding purposes.

SIGNIFICANCE STATEMENT

This study discovered the cytological effects of gamma radiation on selected mutants of wheat (*Triticum estivum* L.) in the third mutation (M3) that can be beneficial for improving crops. This study will help the researchers to uncover the critical areas of the usefulness of gamma radiation in improving crops that many researchers continue to explore.

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