

## INDUCED MUTATIONAL VARIABILITY BY GAMMA-RADIATION OR ETHYLEMETHANE SULPHONATE AND THEIR COMBINATIONS IN PEA *Pisum sativum* L.

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### ABSTRACT

Seeds of the pea cultivar Master B were irradiated with different doses of gamma-rays (0, 2.5, 5, 10, 15, 20, 25 and 30 KR), another portions of the seeds were treated with different levels of EMS (0.0, 0.025, 0.05, 0.1%). Also, there were combined treatments using the two mutagenic agents. The results of this investigation showed that the  $M_2$ -plants exhibited different degrees of reduction in growth in comparison with those of the untreated control plants. In  $M_2$ , the mutagenic doses significantly increased the mean value for plant height, number of pods per plant and seed yield per plant. However, the results did not show additional variation for number of branches per plant and number of seeds per pod. In  $M_2$ -generation, 107 different mutant lines were selected.

### INTRODUCTION

Plant breeding is nothing more than controlled selection. The degree to which selection could be controlled by plant breeder is dependent upon the amount and kind of genetic variation available in the breeding population. Theoretically, this limitation could be overcome either by inducing mutations, or outcrossing, or by a combination of both techniques. The effects of mutagenic agents be measured more readily in homozygous population. Mutants could be used directly to establish a new variety. This approach is time-saving as compared with the other breeding methods (Gaul, 1966). Selection of mutants could start from the  $M_2$ -generation. Selection in  $M_2$ -generation is preferable, since it will imply the highest chance to find the desired mutation among the small irradiated population.

Gamma-rays and ethylemethane sulphonate (EMS) have been used to improve plant characters and increasing genetic variability in several varieties of pea by many investigators such as Balint (1968), Hussein *et al.* (1974) and Kumar *et al.* (1981).

Therefore, the present study was undertaken to evaluate the effects of different gamma-rays and EMS doses on the  $M_2$ -generation in inducing new variability for some important traits in pea. These information may help in the planning of more efficient breeding programs. It is important to mention that pea is a self-pollinating plant and has a narrow spectrum of natural phenotypic variation.

### MATERIALS AND METHODS

Pea seeds *Pisum sativum* L. from Master B cultivar were exposed to different doses of gamma-rays, i.e., 2.5, 5, 10, 15, 20, 25 and 30 KR.

Gamma-rays used in this study was generated from the cobalt-60 source at the Middle Eastern Regional Radioisotope Center of the Arab Countries, Dokki, Cairo, Egypt. Dry seeds of pea cultivar were treated with freshly prepared aqueous solution of ethylmethane sulphonate (EMS). The concentrations at which the mutagen was applied to the seeds were 0.0, 0.025, 0.05 and 0.1% for 12 hours at room temperature. Irradiated seeds in the three doses 25, 5 and 10 KR of gamma-rays were divided into three portions, each one was soaked for 12 hours in 0.025, 0.05 and 0.1% of EMS. While irradiated seeds at high doses of gamma-rays (15, 20, 25 and 30 KR) were not treated by EMS. Treated and non-treated seeds (control) were sown in four replications using a randomized complete blocks design. These planted treated plant have been named M<sub>1</sub>-plants which gave the M<sub>2</sub>-seeds at the end of the season.

M<sub>2</sub> seeds from the individual M<sub>1</sub>-plants from each treatment were sown as a family on November 1<sup>st</sup> 2000/01 and 2001/02 seasons under field conditions, as well as one row of untreated seeds as a control for each ten families of treated seeds.

During the growth period, macromutations such as dwarf, vigorous plants, early or late mature plants, and other morphological changes were harvested separately to give rise to M<sub>3</sub>-families.

At harvest, seed yield and its components were measured for every plant from each family to discover the occurrence of M<sub>2</sub>-micromutations. Data were recorded on plant height (cm), number of branches, pods and seed yield per plant and number of seeds per pod. The statistically analyzed by calculating minimum value, maximum value (range of variability), the man of treatment ( $\bar{X}$ ) variance ( $S^2$ ), standard error (S.E), coefficient of variation (C.V.%), the ratio of coefficient of variation of treatment and the control (C.V.% of treatment / C.V.% of control) to determine the relative variability induced by gamma-rays and ethylmethane sulphonate.

## RESULTS AND DISCUSSION

### Plant height

As shown in Table 1, plant height was reduced as the dose of gamma-rays or EMS concentrations was increased. On the other hand, most treatments of both mutagens induced variability higher than the control. The measurements of variation (range of variability and coefficient of variation) in the treated plants were higher than those of the control plants.

From the above results, it is clear that both gamma-rays and EMS concentrations were able to create and extend an additional variation in plant height of the M<sub>2</sub>-generation. With regard to the control, the present variability could be due to environmental conditions (Hussein *et al.*, 1974). Therefore, the increments in variability in the M<sub>2</sub>-irradiated generation could be due to genetical variation affecting the plant height. This means more opportunities for the selection of the desirable plant height.



### **Number of branches/plant**

Data presented in Table 2 show that the estimates for number of branches per plant under the doses of gamma-rays and EMS were not significantly variable in spite of the appearance of some differences among the treatments means for this trait.

This response of number of branches per plant to radiation, might be the balance between the stimulating effect of the lower doses of mutagens and inhibiting effect of the higher doses (Badr *et al.*, 2000). Also, the depression effect of the high mutagen doses on vegetative traits may be attributed to the active disturbances of some enzyme involved in the synthesis of growth (Abd El-Rahman, 2000).

### **Number of pods per plant:**

Data presented in Table 3 show that in the cultivar Master B in the first season for number of pods per plant was unaffected by the different mutagenic treatments in  $M_2$ -generation. The range of variability for the mutagenic plants appeared similar to that of the control. In the second season the concentration of 0.1% EMS produced the highest (11.0), range of variability for the mutagenic treatments which was wider than the control.

This result is in agreement with that reported by Omar (1995) who found, on *Gomphrena globosa* L. that in  $M_2$ -generation of two seasons the differences among the different doses (0, 5, 10, 15, 25 and 30 KR) of gamma-rays were not significant considering number of inflorescences per plant.

### **Number of seeds/pod:**

Data concerning the number of seeds per pod in Table 4 show that the single mutagenic treatments did not change the number of seeds per pod in both seasons. Therefore, all treatment means had nearly the same value of the number of seeds per pod, and the ranges of variability for irradiated plants were similar to that of the control plants.

This result is in agreement with that reported by Marwan *et al.* (1974) who found that number of pods/plant, seeds/pod and 100-seed weight were positively correlated with seed yield in  $M_2$ -generation after the irradiation of the pea seeds with gamma-rays.

### **Seed yield**

The response of seed yield per plant to mutagenic treatments was somewhat differed in the cultivar Master B. In the first season, seed yield per plant was decreased with the increasing of mutagenic doses. But in the second season, all plants derived from all doses produced similar values of seed yield. Only the dose of 5 KR was able to increase the range of variability in the  $M_2$ -generation compared with the other treatments as presentd in Table 5.

Table 1: Effect of single and combined treatments of gamma-rays and EMS on micromutation in M<sub>2</sub>-generation on plant height of pea cultivar Master B in 2000/01 and 2001/02 seasons.

Treatment	2000/01					2001/02						
	No. of plants	Min.	Max.	X ± S.E.	C.V.%	C.V. treat cv. cont.	No. of plants	Min.	Max.	X ± S.E.	C.V.%	C.V. treat cv. cont.
2.5 KR	381	30	65	46.6±0.39	6.14	0.85	405	39	77	55.7±0.39	5.07	0.83
5 KR	330	30	75	44.5±0.43	6.75	0.93	360	35	76	57.3±0.53	5.44	0.89
10 KR	295	30	70	43.0±0.42	7.30	1.01	391	42	75	54.7±0.43	5.33	0.87
15 KR	276	20	70	45.5±0.45	6.92	0.96	294	35	75	54.6±0.33	5.12	0.84
20 KR	173	20	75	44.7±0.45	7.79	1.08	182	30	85	56.8±0.49	5.94	0.94
25 KR	46	20	65	43.1±0.39	7.35	1.02	52	30	85	58.0±0.42	6.45	1.06
30 KR	37	25	65	42.6±0.41	7.49	1.03	47	35	74	56.0±0.45	5.24	0.86
0.025% EMS	358	20	70	42.5±0.43	7.42	1.02	319	15	80	52.8±0.65	7.08	1.16
0.05% EMS	354	30	70	41.1±0.40	7.30	1.01	362	40	92	62.1±0.49	5.37	0.88
0.1% EMS	315	20	60	43.9±0.42	7.07	0.98	323	42	97	60.9±0.61	5.83	0.96
2.5 KR+0.025% EMS	392	10	75	45.1±0.47	7.93	1.10	410	35	88	57.6±0.39	6.27	1.03
2.5 KR+0.05% EMS	432	20	70	43.0±0.43	5.67	0.78	412	31	81	54.5±0.33	6.07	1.00
2.5KR+0.1% EMS	460	20	70	47.0±0.40	6.23	0.86	423	22	75	55.3±0.38	5.98	0.98
5KR+0.025%EMS	419	30	70	46.4±0.38	6.21	0.86	430	32	80	65.0±0.30	5.18	0.85
5KR+0.05%EMS	388	25	60	47.0±0.35	5.69	0.79	376	42	83	57.7±0.43	5.48	0.90
5KR+0.1%EMS	422	15	70	44.6±0.41	7.51	1.04	385	32	92	58.0±0.34	5.70	0.93
10KR+0.025%EMS	281	25	70	42.8±0.38	7.19	0.99	364	37	95	56.1±0.40	5.87	0.96
10KR+0.05%EMS	265	30	70	44.9±0.46	8.38	1.16	342	31	70	54.5±0.46	5.50	0.90
10KR+0.1%EMS	243	25	70	46.4±0.41	7.19	0.99	276	25	76	56.2±0.36	5.35	0.88
Control	365	15	60	41.7±0.40	7.24	1.00	354	42	79	54.1±0.42	6.10	1.00



Table 2: Effect of single and combined treatments of gamma-rays and EMS on micromutation in M<sub>2</sub>-generation on number of branches per plant of pea cultivar Master B in 2000/01 and 2001/02 seasons.

Treatment	2000/01					2001/02						
	No. of plants	Min.	Max.	X ± S.E.	C.V. %	C.V. treat cv. cont.	No. of plants	Min.	Max.	X ± S.E.	C.V. %	C.V. treat cv. cont.
2.5 KR	381	0	4	0.2±0.11	409.01	1.02	405	0	3	1.0±0.13	96.38	0.57
5 KR	330	0	2	0.1±0.09	606.38	1.52	360	0	3	1.0±0.16	94.38	0.58
10 KR	295	0	6	0.5±0.15	225.74	0.56	391	0	3	0.8±0.14	118.16	0.71
15 KR	276	0	7	0.4±0.16	281.61	0.70	294	0	5	0.9±0.13	116.39	0.70
20 KR	173	0	3	0.2±0.10	386.00	0.97	182	0	2	0.9±0.12	95.52	0.57
25 KR	46	0	4	0.3±0.10	283.26	0.71	52	0	4	0.8±0.12	128.66	0.78
30 KR	37	0	4	0.2±0.10	403.34	1.01	47	0	4	1.0±0.17	108.06	0.65
0.025 % EMS	358	0	2	0.2±0.90	341.25	0.85	319	0	2	0.6±0.15	140.28	0.84
0.05 % EMS	354	0	3	0.2±0.10	379.38	0.95	362	0	2	0.5±0.12	157.89	0.95
0.1 % EMS	315	0	3	0.2±0.10	378.31	0.95	323	0	3	0.7±0.14	118.15	0.71
2.5 KR+0.025% EMS	392	0	4	0.2±0.10	393.94	0.99	410	0	6	1.1±0.12	100.63	0.60
2.5 KR+0.05% EMS	432	0	3	0.2±0.10	386.69	0.97	412	0	4	0.9±0.10	107.50	0.64
2.5KR+0.1% EMS	460	0	3	0.1±0.10	723.99	1.81	423	0	6	0.8±0.12	130.38	0.78
5KR+0.025%EMS	419	0	3	0.2±0.10	388.19	0.97	430	0	4	0.6±0.09	147.38	0.88
5KR+0.05%EMS	388	0	2	0.1±0.80	647.09	1.62	376	0	3	0.8±0.12	107.85	0.65
5KR+0.1%EMS	422	0	3	0.2±0.10	393.87	0.99	385	0	3	0.6±0.09	144.93	0.87
10KR+0.025%EMS	281	0	3	0.2±0.09	375.69	0.94	364	0	4	0.7±0.12	134.94	0.81
10KR+0.05%EMS	265	0	3	0.2±0.09	377.99	0.95	342	0	2	0.7±0.13	117.83	0.71
10KR+0.1%EMS	243	0	3	0.2±0.09	377.10	0.94	276	0	7	1.1±0.13	96.31	0.58
Control	365	0	4	0.2±0.11	399.74	1.00	354	0	3	0.5±0.11	166.9	1.00

Table 3: Effect of single and combined treatments of gamma-rays and EMS on micromutation in M<sub>2</sub> -generation on number of pods per plant of pea cultivar Master B in 2000/01 and 2001/02 seasons.

Treatment	2000/01						2001/02					
	No. of plants	Min.	Max.	X ± S.E.	C.V.%	C.V. treat cv. cont.	No. of plants	Min.	Max.	X ± S.E.	C.V.%	C.V. treat cv. cont.
2.5 KR	381	2	10	3.9±0.19	34.82	1.03	405	3	14	6.9±0.24	25.08	0.96
5 KR	330	1	23	3.6±0.25	49.09	1.45	360	1	17	7.9±0.33	25.00	0.95
10 KR	295	1	14	3.5±0.21	44.35	1.31	391	2	14	6.4±0.25	26.51	1.01
15 KR	276	1	14	3.5±0.22	43.81	1.29	294	3	15	6.8±0.21	25.39	0.97
20 KR	173	1	10	3.3±0.15	36.80	1.09	182	2	15	6.1±0.25	27.99	1.07
25 KR	46	2	11	3.3±0.14	34.33	1.01	52	2	18	6.5±0.21	29.30	1.12
30 KR	37	1	18	3.4±0.19	43.18	1.27	47	2	15	6.5±0.30	29.66	1.13
0.025 % EMS	358	1	5	2.9±0.12	29.32	0.87	319	1	12	4.7±0.28	34.16	1.30
0.05 % EMS	354	1	7	3.2±0.13	29.57	0.87	362	2	16	5.6±0.24	29.41	1.12
0.1% EMS	315	2	12	3.5±0.16	34.41	1.02	323	3	16	11.0±0.64	31.18	1.19
2.5 KR+0.025% EMS	392	1	10	3.8±0.19	38.70	1.14	410	3	30	7.3±0.23	29.13	1.11
2.5 KR+0.05% EMS	432	1	12	3.7±0.19	38.44	1.13	412	2	18	6.5±0.19	29.71	1.13
2.5KR+0.1% EMS	460	2	10	4.2±0.18	31.15	0.92	423	1	13	6.6±0.21	27.27	1.04
5KR+0.025%EMS	419	1	11	3.9±0.17	33.47	0.99	430	2	15	6.3±0.18	27.78	1.06
5KR+0.05%EMS	388	2	12	4.3±0.18	32.43	0.96	376	2	17	7.0±0.24	25.12	0.96
5KR+0.1%EMS	422	1	8	3.1±0.13	32.89	0.97	385	2	18	6.2±0.18	27.88	1.06
10KR+0.025%EMS	281	1	6	3.0±0.12	31.64	0.93	364	1	24	6.0±0.22	30.32	1.16
10KR+0.05%EMS	265	1	6	3.1±0.12	32.19	0.95	342	1	12	5.4±0.24	29.55	1.13
10KR+0.1%EMS	243	1	6	3.1±0.12	31.70	0.94	276	1	14	7.2±0.22	25.27	0.97
Control	365	1	5	3.2±0.14	33.88	1.00	354	2	8	4.7±0.16	26.18	1.00



Table 4: Effect of single and combined treatments of gamma-rays and EMS on micromutation in M<sub>2</sub>-generation on number of seeds per pod of pea cultivar Master B in 2000/01 and 2001/02 seasons.

Treatment	2000/01						2001/02					
	No. of plants	Min.	Max.	X ± S.E.	C.V.%	C.V. treat cv. cont.	No. of plants	Min.	Max.	X ± S.E.	C.V.%	C.V. treat cv. cont.
2.5 KR	381	3	9	7.4±0.21	20.18	1.41	405	3	9	5.8±0.17	20.69	0.95
5 KR	330	2	9	7.3±0.23	22.43	1.57	360	2	9	5.8±0.23	23.93	1.10
10 KR	295	3	8	6.3±0.22	25.44	1.78	391	3	8	6.3±0.18	18.91	0.87
15 KR	276	2	9	6.8±0.22	22.49	1.57	294	3	8	5.6±0.17	24.95	1.15
20 KR	173	2	8	6.6±0.18	21.71	1.52	182	2	8	5.8±0.19	22.58	1.04
25 KR	46	2	8	7.8±0.15	15.42	1.08	52	2	8	5.7±0.15	23.47	1.08
30 KR	37	2	8	8.0±0.14	13.44	0.94	47	3	8	5.0±0.18	22.78	1.05
0.025 % EMS	358	3	9	7.9±0.19	17.64	1.24	319	2	8	5.2±0.29	25.29	1.16
0.05 % EMS	354	3	9	7.7±0.17	16.46	1.15	362	3	9	6.3±0.18	19.68	0.91
0.1 % EMS	315	3	9	8.0±0.16	14.50	1.02	323	4	8	5.7±0.21	21.22	0.98
2.5 KR+0.025% EMS	392	2	9	7.3±0.19	20.14	1.41	410	2	9	5.9±0.23	24.45	1.13
2.5 KR+0.05% EMS	432	2	8	6.7±0.21	24.19	1.69	412	2	8	5.8±0.18	21.37	0.98
2.5KR+0.1% EMS	460	2	9	7.4±0.20	19.80	1.39	423	4	9	5.4±0.19	23.67	1.09
5KR+0.025%EMS	419	2	8	7.7±0.17	16.31	1.14	430	1	8	6.4±0.16	17.17	0.79
5KR+0.05%EMS	388	2	8	7.8±0.18	17.76	1.24	376	3	8	5.1±0.18	25.69	1.18
5KR+0.1%EMS	422	3	9	6.8±0.16	19.61	1.37	385	3	8	6.0±0.17	20.20	0.93
10KR+0.025%EMS	281	3	9	7.5±0.17	18.63	1.30	364	3	8	6.3±0.16	20.38	0.94
10KR+0.05%EMS	265	2	8	7.4±0.17	18.36	1.29	342	3	8	5.5±0.15	24.91	1.15
10KR+0.1%EMS	243	3	9	7.5±0.14	15.49	1.08	276	3	9	5.4±0.15	23.51	1.08
Control	365	5	9	8.2±0.16	14.28	1.00	354	5	9	5.8±0.16	21.71	1.00

Table 5: Effect of single and combined treatments of gamma-rays and EMS on micromutation in M<sub>2</sub>-generation on seed yield per plant of pea cultivar Master B in 2000/01 and 2001/02 seasons.

Treatment	2000/01					2001/02						
	No. of plants	Min.	Max.	X ± S.E.	C.V.%	C.V. treat cv. cont.	No. of plants	Min.	Max.	X ± S.E.	C.V.%	C.V. treat cv. cont.
2.5 KR	381	1.11	7.29	3.7±0.17	33.09	1.00	405	0.9	19.2	6.0±0.22	27.09	0.94
5 KR	330	0.16	6.49	3.0±0.18	42.48	1.28	360	1.0	15.0	7.4±0.36	29.08	1.01
10 KR	295	0.00	5.54	2.5±0.16	48.37	1.46	391	0.8	24.4	6.1±0.23	25.08	0.87
15 KR	276	0.00	5.23	2.9±0.17	39.92	1.20	294	1.0	13.5	5.9±0.21	29.86	1.04
20 KR	173	0.32	6.34	2.8±0.14	37.90	1.14	182	1.4	25.0	5.5±0.24	29.99	1.04
25 KR	46	1.11	7.29	3.4±0.13	30.91	0.93	52	0.8	19.6	5.8±0.22	32.79	1.14
30 KR	37	1.11	7.13	3.4±0.15	34.16	1.03	47	1.6	22.2	5.1±0.27	35.13	1.22
0.025% EMS	358	0.32	5.86	3.2±0.15	34.19	1.03	319	1.6	25.5	4.0±0.28	40.89	1.42
0.05% EMS	354	0.32	6.81	3.3±0.14	32.83	0.99	362	0.3	13.3	5.5±0.25	30.14	1.04
0.1% EMS	315	0.79	8.40	3.5±0.16	33.64	1.01	323	1.3	26.1	6.0±0.29	28.24	0.98
2.5 KR+0.025% EMS	392	0.95	10.77	3.0±0.17	42.78	1.29	410	1.0	14.3	6.0±0.31	32.59	1.13
2.5 KR+0.05% EMS	432	0.16	6.65	3.7±0.13	26.97	0.81	412	2.1	10.9	5.4±0.25	30.88	1.07
2.5 KR+0.1% EMS	460	0.79	8.87	4.1±0.19	34.80	1.05	423	0.3	18.9	4.7±0.46	34.76	1.2
5KR+0.025%EMS	419	1.27	9.82	3.6±0.16	32.79	0.99	430	1.6	27.7	6.1±0.26	28.28	0.98
5KR+0.05%EMS	388	1.42	6.65	3.5±0.19	40.55	1.22	376	1.0	15.1	5.4±0.21	28.27	0.98
5KR+0.1%EMS	422	0.16	4.44	2.7±0.12	36.42	1.10	385	1.0	16.3	6.2±0.24	27.43	0.95
10KR+0.025%EMS	281	0.79	5.70	8.9±0.85	76.75	2.32	364	1.1	14.3	6.0±0.22	30.57	1.06
10KR+0.05%EMS	265	1.11	6.49	6.0±0.06	80.03	2.41	342	1.9	19.0	5.0±0.23	30.94	1.07
10KR+0.1%EMS	243	1.42	6.34	3.2±0.13	33.57	1.01	276	0.8	11.9	6.0±0.20	27.40	0.95
Control	365	0.63	6.34	3.7±0.17	33.15	1.00	354	0.9	11.4	4.4±0.16	28.85	1.00



This results is agreement with Mehandjiev (1970) on pea who found that the combined effects of irradiation and chemical mutagens exceeded the additive effects on mutation rate gave less common mutations than those obtained by treatment with the physical or chemical mutagens alone and also yielded a wider spectra of economically useful mutation. Sanaev *et al.* (1976) on pea found that treated with gamma-rays (5-10 KR) and soaked 0.05-0.1% solutions of cyclophosphane after irradiated with gamma rays the highest percentage of mutants in the M<sub>2</sub>-generation was obtained after treatment with 10 KR and 0.1% cyclophosphane and selected three mutants with more seeds per pod, larger seeds and plant height than the initial form. Moustafa (1987) on cowpea found that irradiation with gamma-rays at doses of 0, 2.5, 5, 7.5, 10 and 15 KR in M<sub>2</sub>-generation 2.5 KR and 5 KR increased the yield and most of its components.

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اختلافات طفورية ومستحدثة بأشعة جاما والإيثيل ميثان سلفونيت والتفاعل المشترك بينهم في نبات البسلة  
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أجرى هذا البحث خلال موسمين متتاليين (٢٠٠٠/٢٠٠١)، (٢٠٠١/٢٠٠٢) بمزرعة كلية الزراعة بكفر الشيخ بهدف دراسة تأثير أشعة جاما والمطفر الكيماوى أيثايل ميثان سلفونيت على نبات البسلة صنف ماستر بى على نباتات الجيل الثانى. ويمكن تلخيص أهم النتائج المتحصل عليها كما يلي:  
١- أظهرت معظم المعاملات المستخدمة من أشعة جاما والمطفر الكيماوى إختلاف فى كل من طول النبات، عدد القرون للنبات، المحصول البذرى للنبات عن الكنترول فى كلا الموسمين.  
٢- لم تحدث إختلافات وراثية فى صفة عدد الافروع للنبات وعدد البذور للقرن نتيجة المعاملات المستخدمة فى كلا الموسمين.