MUTATION FREQUENCY IN MUNGO *(PHASEOLUS RADIATUS* L.) AFTER TREATMENT WITH AN ALKYLATING CHEMICAL MUTAGEN

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ABSTRACT

Dormant seeds of green mungo *(Phaseolus radiatus* L.) were treated with aqueous solution of EMS at concentrations of 0.02M to 0.06M with phosphate buffer for periods of 6 to 24 hours at approximately 30° C. Seeds soaked in distilled water and buffer solution were used as controls.

The seeds were grown on moist blotting paper for germination percentage and seedling height measurements after seven days. The M_1 seedlings were transplanted in field plots in a dose-to-row plan with two replications for determination of the frequency of somatic mutations, percentage of field survival at flowering stage, and seed-set. The M_2 progenies were grown for determination of types and frequency of chlorophyll-deficient mutations as an index of the degree of genetic change due to the mutagen.

In general, a direct dose-effect relationship was obtained for the various biological criteria employed to measure the effectivity of the mutagen. These are percentage of germination after treatment, seedling height, somatic mutations, seed-set and germinal mutations.

Three types of induced chimeral sectors on leaves of M_1 plants such as pale green, yellow green and yellow occurred with a frequency ranging from 4.65 to 31.03 per cent. Four types of chlorophyll-deficient seedling mutations were obtained, i.e., virescent, chlorina, xantha and albina with a frequency ranging from 0.23 to 7.55 per cent on the seedling basis and 2 to 26 per cent on the pod basis.

I. INTRODUCTION

The mutagenic effects of some chemical compounds have been demonstrated by various investigators on different organisms. Their effects on lower organisms [1, 33, 36, 37] have led to their wide use in the mutation breeding of higher plants. Some have been used successfully for the induction of mutations in barley, corn, rice, tomato, peas and wheat [6, 11, 13, 15, 16, 30, 38, 46, 54].

Ethyl methanesulfonate, in particular, is one of the mutagenic agents now widely used for producing genetic changes due to the high mutation rates and few gross chromosomal aberrations obtained in treated lines [3, 43, 55, 56]. In addition, some workers [12, 24, 54, 55, 56] have reported a mutation spectrum different from that induced by other chemicals and radiations. A study on the frequency of mutations induced by the EMS in mungo seeds may provide useful information on the fundamental process and on its value in mutation breeding.

Objects of this study were: (1) to determine the response of mungo seeds to various doses of EMS at varying period of soaking, and (2) to test the mutagenicity of this chemical in this species under the conditions of the experiment.

II. REVIEW OF LITERATURE

It is known from results of studies by various investigators that EMS is an efficient mutagen, i.e., with a high rate of mutations and relatively few gross chromosomal aberrations. Konzak *et al*[5]. emphasized that while some chemical mutagens are fully radiomimetic, giving essentially the same pattern of mutation and gross chromosomal aberrations as the ionizing radiations, EMS produces more mutations and fewer gross chromosomal aberrations.

Bhatia and Van Der Veen[3] reported that EMS has a low toxicity in *Arabidopsis thaliana* based on a large scale M_1

survival-fertility data high survival to flowering and high fertility compared with the control. According to the same investigators EMS seems rivalled only by nitroguanidine in efficiency and low level of undesirable physiological effects. Zannone[5,6] also noted that in two line of *Vicia sativa* L. the EMS spectrum had 12 types of chlorophyll mutations, a range wider than those of ethylene imine and X-rays.

The effects of EMS on the M_1 or treated generation have been used not only to measure the biological response of seeds but also to obtain possible relationships between such effects and genetic changes. A correlation between M_2 mutation rate and frequency of leaf spot in chemical-treated peas was reported[18]. The relationship was so high that he suggested taking seeds only from M_1 plants showing the highest number of leaf spots in order to obtain a high mutation rate in the M_2 generation.

As in other investigations, physiological and morphological changes in the M_1 or treated generation as compared with the control have been used to determine the degree of damage or effects by a given mutagen while the fertility and occurrence of chlorophyll mutations in the second or M_2 generation have been employed to test the mutagenicity of a given treatment. The use of seed as a valid index of the frequency of chromosomal aberrations has been widely accepted on account of the high correlation between these two mutagenic effects[31,52].

For the purpose of evaluating the mutagenicity of EMS, studies were made to compare its effects with those of known radiations on seeds like X-rays, gamma radiations and neutrons. Results of early investigations have been reviewed by various workers[1,9,29]. These reports show that although the response of seeds to these mutagens vary from species to species, a general dose-effect relationship was obtained for most of the biological criteria like seed viability, growth retardation and visible chromosome aberrations. Reports[44,55] showed that EMS yielded approximately seven times as many chlorophyll mutants than X-rays and gamma rays which equalled each other, approximately five times as many mutants as all the radiations, a relatively high percentage of

which was due to neutrons. The conclusion was that EMS had the most promising results and neutrons were next best but have the advantage of producing much less sterility.

Studies have also shown the high efficiency of EMS as a mutagenic agent. Favret[15] studied the mutation frequency of four genes for albinism in barley by means of treatments with X-rays and EMS. All four genes mutated with similar frequency after X-ray treatment but with EMS only one gene mutated. It was concluded that EMS had a selective mutagenic effect and is a more effective mutagen than X-rays. Gaul[18] likewise reported a high mutagenic efficiency of EMS in barley. He found it to be 3 to 8 times higher than that of X-rays. He also believed that EMS has a relatively lower toxic effect and higher genetic effect than X-rays. Moreover, Heslot[24] found many more morphological mutations per chlorophyll mutation after EMS treatment resulted in much lower chromosome breakage but caused a higher M_1 sterility[55].

In the work of Jacobsen[28] many plants developed from mutagenically treated seeds were chimeric in nature and consisted of both mutated and non-mutated cells. Consequently, flower shoots and whole group of shoots can become either mutated or non-mutated for one or more layers of cells. If a male or female tissue is derived from a mutated cell layer in a certain part of the plant, mutated offsprings will be segregated after autogamy.

Relatively recent techniques on mutagenic treatment of seeds have been given more valid determination on account of the control of treatment conditions for which suitable laboratory methods have been devised[5, 17, 27, 30, 41]. Response of seeds to mutagenic treatments have been found to be influenced largely by environmental conditions like temperature pH, oxygen concentration, and seed moisture content. Hence, measurements of radiations and chemical agents have been done with the said conditions under control[5, 42]. Pre-and post-treatments have been found to increase viability, growth rate, M_1 fertility and mutation frequency by a factor of eight[30, 42]. Nilan and his co-

workers[42] proposed that the effects caused by radiation and by chemical mutagens can be measured in terms of several criteria such as M_1 plant survival following treatment, frequency of seedling flecking and of chlorophyll-deficient chimera in the M_1 plants, frequency of chromosomes bridges and fragments in the shoot tips of mutagen-treated seeds chromosome translocation and inversions in meiosis in M_1 plants and the frequency of chlorophyll-deficient seedling mutations among the M_2 progenies.

In mungo seeds the action of EMS has been previously tested[51]. A linear dose-effect relationship was obtained for various types of biological responses. The mutation spectrum consisted of more viables than lethals.

III. MATERIALS AND METHODS

The experiments were done at the Botany Garden, University of the Philippines, Quezon City, from January to July, 1966 and from February to September, 1967. Mutagen treatments were done on January 15-16, 1966 and on February 25-26, 1967 at the Botany Greenhouse laboratory.

One thousand selected seeds from a pureline of green mungo *(Phaseolus radiatus* L., Family Leguminosae) (Plate I) were placed in plastic vials each containing 50 seeds and soaked for two hours in distilled water before mutagen treatment. After pre-treatment, the seeds were allowed to stand for a few minutes to remove excess water and then placed in aqueous solutions of EMS at concentrations of 0.02M to 0.06M with phosphate buffer for periods of 6 to 24 hours. Seeds soaked only in distilled water and buffer solution were used as controls. The treatments were done at a temperature of approximately 30±1°C. The EMS stocks were purchased from Distillation Products Industries, Rochester 3, New York, U.S.A. in December, 1965 and in January, 1967.

Nitrogen gas was bubbled through the EMS solutions after 18 hours to minimize high acidity. As post-treatment, the seeds were rinsed briefly in running water and soaked in

distilled water for four hours with hourly changes to wash away the excess chemical.

The seeds were first grown on moist blotting paper in petri dishes under room conditions. Seedling height was measured seven days after planting. The seedlings were transplanted in field plots in a dose-to-row plan with two replications. Distances of planting were 30 centimeters between the rows and 18 centimeters in the row.

The plants were scored for somatic mutations in the form of chimeral leaf sectors after planting. Upon maturity, the first five maturing pods from each plant were harvested and scored for M_1 fertility as determined from seed-set per plant.

One hundred pods per dose from the first five maturing pods per plant were selected at random for M_2 seedling mutation study. The M_2 seeds were planted in seed flats in a pod-to-row test for segregation of chlorophyll mutations. The types and frequencies of chlorophyll mutations in the M_2 generation were determined. The mutagenic efficiency of each treatment was calculated.

IV. RESULTS AND OBSERVATIONS

Percentage of Germination. In general, only a slight damage was caused by the chemical in mungo seeds. As shown in Table, I, marked reductions in seed germination were obtained only at an EMS dose of 0.06M, 24 hours. No such reductions were obtained at lower doses and in both the water and buffer controls. At the lethal dose, approximately 54 per cent failed to germinate evidently due to toxicity of the EMS solution. The data show that the LD^{50} of EMS on this species is approximately 0.06M, 24 hours, at a temperature of 30° C.

Seedling height. The effect of EMS on seedling height is shown in Table II. Based on the controls, seedling growth reductions were obtained at doses at 0.04M, 24 hours and 0.06M, 18 hours and 24 hours.

A dose of 0.04M, 18 hours reduced seedling height by approximately 9.8 centimeters or 76.59 per cent of the control; 0.04M, 24 hours, 8.5 centimeters or 62.04 per cent; and 0.06M, 24 hours, 12.2 centimeters or 89.05 per cent.

These differences were all significant at the 5 per cent level. A general dose-effect relationship was obtained for reductions in seedling height in the different treatments.

Somatic mutations. The somatic mutations were manifested as chlorophyll-deficient areas for sectors on both young and mature leaves. The three types of somatic mutations found in the M_1 plants were pale green, yellow green and yellow sectors.

The types and frequency of M_1 plants bearing at least one chimeral leaf are shown in Table III. The frequency of somatic mutations on the M_1 plant basis ranged from 4.65 to 31.03 per cent. Although the occurrence of somatic change was more or less random, the doses were in general effective in causing this type of change since almost all the treatments had at least one chimerical plant.

M_1 survival. The percentage of surviving plants of flowering time in the different treatments are shown in Table IV. The significant reductions in M_1 survival was obtained at doses of 0.06M, 18 hours and 0.04M, 24 hours giving 70.21 per cent and 53.19 per cent, respectively. Although plant survival is subject to many environmental factors from planting to flowering age, the data show that such effects did not cause any significant reductions in the other treatments.

The seed-set data are shown in Table V. The mean number of seeds per pod in the control ranged from 9.18 to 10. 61 while in the treated lots, the mean number of seeds per pod ranged from 5.12 to 10.38 which represents 64.81 to 100 per cent of the control. The seed-set in the treated lots ranged from 59.18 to 91.17 per cent.

Reductions in seed-set were obtained at doses of 0.06M, 12 hours; 0.06M 18 hours and 0.04M, 24 hours. Pods from these doses had 1 to 7 aborted ovules or empty locules while normal pods had 0 to 3 empty locules.

The seed-set at 0.06M, 12 hours was found to be 69.42 per cent which was 77.67 per cent of the control; at 0.06M, 18 hours, 59.23 or 69.61 per cent of the control; and 0.04M, 24 hours, 59.18 per cent or 64.81 per cent of the control. In general, the data on seed-set reduction followed a linear dose-effect relationship.

Germinal mutations. Four types of chlorophyll deficient mutations were observed in the M_2 generation, i.e., virescent (pale green), chlorina (yellow green), xantha (yellow), and albina (white).

Mutation frequency (Pod basis). As the M_2 seedlings were grown in a pod-to-row plan, segregation in an M_1 pod was indicated by the occurrence of a chlorophyll-deficient seedling. All the EMS doses yielded at least one mutant-bearing pod. The overall mutation rate on the pod basis ranged from 2 to 26 per cent in all the treatments (Table VII).

Mutagenic efficiency. The mutagenic efficiency of the various doses of EMS are given in Table VI. Those values were computed as the ratio of M_2 seedling mutation frequency per 100 M*1* pods to percentage of lethal plants in the M_1 treated generation. The highest mutagenic efficiency of EMS on mungo is at 0.06M, 12 hours which gave 1.50 efficiency.

V. DISCUSSION

Based on the foregoing results, the various EMS treatments appear to be very effective in causing biological changes in mungo. The general dose-effect relationship obtained for each of the various criteria for biological effects such as percentage of germination, seedling height and seed-set shows that such changes were primarily due to the chemical treatments. These biological effects of the chemical serve as an index of its general usefulness as a mutagenic agent.

In most mutation studies, the percentage of germination of treated seeds has been used as a measure of the initial seed damage or toxicity of the treatment[52], £°°I viability may be a profound effect on the perpetuation of mutant cells to the reproduction stage. Seedling height has been highly correlated with various somatic changes and mutation frequency[31, 42]. The adverse effect of the treatment on seedset has been attributed to chromosomal aberrations[31,50] resulting in ovule and pollen abortions.

The mutagenic effect of EMS has been measured in the form of somatic mutations or leaf chimera and as germinal mutations in the form of chlorophyll deficiency progeny. In general, approximately 5 to 27 per cent of the plants had a chimeral leaf. The origin of chimeral sectors has been described[7] and traced to the main shoot apex of the embryo. According to this worker and other investigators[18, 55] the occurrence of a chimeral sector is dependent on the number of intrasomatic conditions like the ontogenetic development of the cell in the shoot apex and the stage of chromosomal division at the mutagenic treatment.

The frequency of germinal mutation in the M_2 progeny is probably the best index of genetic change by a given mutagen and has been used for this purpose in both basic and applied induced mutation studies[17, 19, 31, 42]. On the M_2 seedling basis, the mutation frequency ranged from 0.23 to 7.55 per cent consisting of approximately 69 per cent virescent, 12 per cent chlorina, 13 per cent xantha and 6 per cent albina while on the M_1 pod basis, the mutation frequency ranged from 2 to 26 per cent.

Compared with treatments in other species, EMS yielded 9 per cent in barley in 0.04M, 5 hours, 30°C[16]; 19 per cent, 10 per cent solution 24 hours, 30°C in Vicia sativa L.[56]; 72 per cent, 0.04 per cent solution, and 24 hours at room temperature in *Pisum sativum*[55].

Treatment with other chemical mutagens like the ethylene-oxide gave 5.8 per cent at 0.3 per cent solution, 10 hours room temperature in barley[10, 11, 12]; ethyleneimine, 27 per cent at 0.04 per cent solution, 2 hours, room temperature in barley[22]; diepoxybutane, 11.3 per cent at 0.2 per

cent solution, 18 hours in corn[32]; diethyl sulfate 3.4 per cent, 0.001M, 2 hours, 30°C in barley[22].

The mode of mutagenic action of EMS on seeds has been studied by several investigators[2, 24, 45]. EMS is an alkylating agent bearing one reactive alkyl group which reacts with DNA leading to the breakage of the linkage between the phosphate and the deoxyribose. Probably the most frequent effect of EMS is the alkylation of guanine base resulting in base transition or base transversion which are believed to be the basic mutational events in the seed[25, 35].

The expression of induced genetic changes in the seed is believed to be largely influenced by a number of intracellular and extracellular conditions during the period of growth[18, 52]. In species where spike or shoot initials have been counted or ranked, it has been possible to relate the size of the chimeral sector and the mutation rate (pod basis) with the number of mutated embryo cells[18]. Thus, chlorophyll-deficient seedling mutations serve as a reliable index of the degree of genetic change in the seed after mutagenic treatment.

The overall usefulness of a given treatment has been determined by its mutagenic efficiency[31]. The most efficient dose appears to be 0.06M, 12 hours giving a mutation rate of 7.55 per cent on the seedling basis and 26 per cent on the pod basis with a survival rate of 75 per cent of the control. This could mean that a useful dose should be only 25 per cent lethal at the time flowering stage in order to be mutagenically effective and efficient. This lethality could perhaps be taken as an index of the rate of the survival of mutation-bearing cells through the growth stage of the M_1 plants.

VI. SUMMARY

1. Seeds of mungo were soaked in 0.02M to 0.06M aqueous solutions of EMS with phosphate buffer at approximately 30°C for 6 to 24 hours. Water-soaked and buffer-soaked seeds from the same stock were used as controls.

2. The seeds were grown on moist blotting paper on petri dishes under room conditions. After a period of seven days, seedling height measurements were obtained and then the plants were transplanted in the field in a dose-to-row plan with two replications at distance of 30 centimeters between the rows and 18 centimeters in the row. Data on somatic mutations and seed-set were obtained.

3. In general, direct dose-effect relationships were obtained for all the criteria observed in the M_1 generation. Significant reductions in seed-set were obtained at 0.06M, 12 hours; 0.06M, 18 hours; and 0.04, 24 hours.

4. The types of somatic mutations observed were pale green, yellow green and yellow sectors. The frequency of M_1 plants having at least one chimeral leaf ranged from 4.65 to 31.03 per cent.

5. Four types of chlorophyll-deficient seedling mutations were observed such as virescent, chlorina, xantha, and albina occurring in varying frequency ranging from 0.23 to 7.55 per cent on the seedling basis and 2 to 26 per cent on the pod basis.

6. The most mutagenic doses appeared to be 0.04M, 24 hours for somatic mutations giving a mutation rate of 31.03 per cent and 0.06M, 12 hours for germinal mutation giving 7.55 per cent on the seedling basis and 26 per cent on the pod basis.

VII. LITERATURE CITED

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TABLE I

Percentage of Germination in Mungo Seeds
After EMS Treatment

	No. of damaged seeds	Germination rate (% of control)
O (H_2O), 6 hours.	0	100
O.O2M , " "	0	100
O.O4M , " "	0	100
O.O6M , " "	2	96
Buffer control, 6 hours	0	100
O (H_2O), 12 hours	1	100
O.O2M , " "	0	100
O.O4M , " "	0	100
O.O6M , " "	3	96
Buffer control, 12 hours	0	100
O (H_2O), 18 hours	0	100
O.O2M , " "	2	96
O.O4M , " "	3	94
O.O6M , " "	5	90
Buffer control, 18 hours	0	100
O (H_2O), 24 hours	0	100
O.O2M , " "	2	96
O.O4M , " "	5	90
O.O6M , " "	27	46
Buffer control, 24 hours	0	100

TABLE II

Mean Heights of Seven Day-Old Mungo Seedling After EMS Treatment

Conc. x Time	Range seedling height (cm.)	Mean Seedling height (cm.)
O (H_2O), 6 hours	7.3 — 20.6	14.7 ± .45
O.O2M , " "	6.9 — 20.0	13.6 ± .55
O.O4M , " "	4.2 — 18.5	12.3 ± .54
O.O6M " "	4.4 — 13.5	8.2 ± .27
Buffer control, 6 hours	3.8 — 20.4	13.8 ± .55
O (H_2O), 12 hours	6.3 — 20.5	14.1 ± .57
O.O2M , " "	5.9 — 17.8	10.6 ± .42
O.O4M , " "	1.0 — 17.8	9.3 ± .44
O.O6M , " "	1.9 — 12.0	6.8 ± .53
Buffer control, 12 hours	1.0 — 18.3	13.8 ± 63
O (H_2O), 18 hours	4.0 — 20.1	12.8 ± .54
O.O2M , " "	3.5 — 14.2	9.1 ± .42
O.O4M , " "	1.0 — 10.4	6.1 ± .44
O.O6M , " "	1.0 — 5.7	3.0 ± .53
Buffer control, 18 hours	2.9 — 18.8	12.9 ± .63
O (H_2O), 24 hours	3.7 — 20.2	13.7 ± .63
O.O2M , " "	3.7 — 15.7	9.7 ± .36
O.O4M , " "	1.0 — 7.1	5.2 ± .18
O.O6M , " "	1.0 — 3.2	1.5 ± .13
Buffer control, 24 hours	3.9 — 19.0	12.2 ± .57

TABLE III

Frequency of Leaf Chimeras In M_1 Mungo Plants
After EMS Treatment

Conc. x Time	No. of plants with at least one chimeral leaf			Total chimeral plants	% chimeral plants
	Pale green	Yellow green	Yellow		
O (H_2O), 6 hours	—	—	—	0	0
O.O2M , " "	—	—	—	0	0
O.O4M , " "	3	—	—	3	7.90
O.O6M , " "	7	—	—	7	14.89
Buffer control, 6 hours	—	—	—	0	0
O (H_2O), 12 hours	—	—	—	0	0
O.O2M , " "	2	—	—	2	4.65
O.O4M , " "	6	3	—	9	19.56
O.O6M , " "	5	1	1	7	18.92
Buffer control, 12 hours	—		—	0	0
O (H_2O), 18 hours	—	—	—	0	0
O.O2M , " "	2	—	1	3	6.98
O.O4M , " "	5	1	1	7	20.59
O.06M , " "		—	—	0	5.66
Buffer control, 18 hours	—	—	—	0	0
O (H_2O), 12 hours	—	—	—	0	0
O.O2M , " "	—	—	—	0	0
O.O4M , " "	9	—	—	9	31.03
O.O6M , " "	—	—	—	—	—
Buffer control, 24 hours	—	—	—	0	0
Total	39	5	4	48	

TABLE IV

Survival of M_1 Mungo Plants
At Flowering After EMS Treatment

Conc. x Time	No. of lethal Plants	Survival (% of control)
O (H_2O), 6 hours	7	100.00
O.O2M , " "	9	95.35
O.O4M , " "	12	88.37
O.O6M , " "	4	100.00
Buffer control, 6 hours	13	86.05
O (H_2O), 12 hours	6	100.00
O.O2M , " "	10	90.91
O.O4M , " "	7	97.72
O.O6M , " "	17	75.00
Buffer control, 12 hours	16	78.64
O (H_2O), 18 hours	3	100.00
O.O2M , " "	10	85.11
O.O4M , " "	20	63.83
O.O6M , " "	36	29.79
Buffer control, 18 hours	13	79.15
O (H_2O), 24 hours	3	100.00
O.O2M , " "	15	74.47
O.O4M , " "	28	46.81
O.O6M , " "	50	0
Buffer control, 24 hours	9	87.23

TABLE V

Seed-set in Mungo Pods
After EMS Treatment

Conc. x Time	Mean number of seeds per pod	Seed-set (% of control)
O (H_2O), 6 hours	9.95	100.00
O.O2M , " "	10.38	100.00
O.O4M , " "	9.54	93.06
O.O6M , " "	8.76	91.51
Buffer control, 6 hours	9.75	97.99
O (H_2O), 12 hours	9.93	100.00
O.O2M , " "	9.24	97.66
O.O4M , " "	7.96	85.21
O.O6M , " "	6.97	77.67
Buffer control, 12 hours	9.44	95.15
O (H_2O), 18 hours	9.18	100.00
O.O2M , " "	9.06	94.31
O.O4M , " "	6.49	78.02
O.O6M , " "	5.12	69.61
Buffer control, 18 hours	10.39	100.00
O (H_2O), 24 hours	10.61	100.00
O.O2M , " "	8.38	87.23
O.O4M , " "	5.63	64.81
O.O6M , " "	—	—
Buffer control, 24 hours	10.57	99.62

*Completely lethal dose.

TABLE VI

Frequency of M_2 Chlorophyll Mutations
(Seedling Basis) and Mutagenic Efficiency of EMS in Mungo

Conc. x Time	Total M_2 seedlings	Mutant-seedlings				Total	Mutation rate (%)	Mutagenic efficiency (Mp/L)*
O (H_2O), 6 hrs.	1,036	—	—	—	—	0	0	0
O.O2M , " "	1,016	2	—	—	—	2	0.23	0.11
O.O4.M , " "	928	3	1	—	—	4	0.39	0.17
O.O6M , " "	845	3	—	1	—	4	0.47	0.50
Buffer control, 6 hrs.	789	—	—	—	—	0	0	0
O (H_2O), 12 hrs.	854	—	—	—	—	0	0	0
O.O2M , " "	976	2	—	—	3	5	0.51	0.25
O.O4M , " "	839	9	3	3	—	15	1.79	1.07
O.O6M , " "	672	38	2	6	5	51	7.55	1.50
Buffer control, 12 hrs.	949	—	—	—	—	0	0	0
O (H_2O), 18 hrs.	880	—	—	—	—	0	0	0
O.O2M , " "	917	5	—	4	—	9	0.98	0.45
O.O4M , " "	489	12	2	3	—	17	3.48	0.42
O.O6M , " "	232	2	4	—	—	6	2.58	0.08
Buffer control, 18 hrs.	897	—	—	—	—	0	0	0
O (H_2O), 24 hrs.	924	—	—	—		0	0	0
O.O2M , " "	785	5	2	1	—	8	1.02	0.27
O.O4M , " "	410	11	2	—	—	13	3.15	0.23
O.O6M , " "	—	—	—	—	—	—	—	—
Buffer control, 24 hrs.	933	—	—	—	—	0	0	0
TOTAL	15,371	92	16	18	8	134		

*Konzak, *et al.*, 1965
Mp = chlorophyll mutant seedlings per 100 M_1 pods
L = percentage M_+ lethality

TABLE VII

Mutation Frequency (Pod Basis) In Mungo After EMS Treatment

Dose	Number of segregating M_1 pods	% Mutation rate
O (H_2O), 6 hours	0	0
O.O2M , " "	2	2
O.O4M , " "	2	2
O.O6M , " "	2	2
Buffer control, 6 hours	0	0
O (H_2O), 12 hours	0	0
O.O2M , " "	3	3
O.O4M , " "	11	11
O.O6M , " "	26	26
Buffer control, 12 hours	0	0
O (H_2O), 18 hours	0	0
O.O2M , " "	6	6
O.O4M , " "	9	9
O.O6M , " "	5	5
Buffer control, 18 hours	0	0
O (H_2O), 24 hours	0	0
O.O2M , " "	6	6
O.O4M , " "	11	11
O.O6M , " "	—	—
Buffer control, 24 hours	0	0