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1. Mutations induced by ethyl methanesulfonate (EMS).

The work reported here involves the use of endosperm marker genes on the short arm of chromosome 9 as a system for testing the mutagenicity of EMS and comparing its effects with those of ionizing radiations in producing chromosome breaks and gene mutations.

Stocks of multiple dominant homozygotes (I Sh Wx) were treated and crossed, as male or female parent, with a corresponding multiple recessive stock (c sh wx). In order to obtain large mutant areas, and to facilitate the establishment of sufficient seed stocks of the induced mutants for further study, EMS and radiation treatments were applied mostly to seed embryos or very young seedlings.

Since EMS becomes hydrolyzed in water over a period of time, and since its chromosome-breaking ability is influenced by impurities in the aqueous solvent, the following precautions were used in preparing EMS solutions. All treatments were begun within an hour after preparation of the solutions. The water used in all experimental procedures was distilled and deionized in order to provide conditions which minimize the production of chromosomal aberrations.

Three methods of treatment with EMS were used:
1) Seed soaking. The surface of the seeds was first disinfected with a mixture of equal portions of 95% ethyl alcohol and 3% hydrogen peroxide. The seeds were then soaked in deionized water at 27° C and bubbled continuously with oxygen for 24 hours. They were then soaked in 0.05 M or 0.025 M aqueous solution of EMS for either 5 hours at 27° C or for 2 to 5 days at 3° C. The rationale for the latter treatments, i.e., for prolonged applications at a cold temperature, was to ensure thorough penetration without chemical disintegration of the mutagen. This was followed by post-incubation in water. In this preliminary report the results of all seed soaking treatments are combined, since no conclusive evidence of significant differences attributable to different methods of soaking is yet available.

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2) Root cutting. Seeds were prepared in the same manner as for the seed soaking treatment, then placed on slanted moist filter paper to ensure growth of straight roots. When the young roots reached 2 to 5 cm in length, the tips were cut off about 0.5 cm from the end. These young seedlings were then put in glass vials so that the root was immersed in a solution of 0.01M EMS, while the seed and shoot remained in air for normal respiration. This treatment was continued for 24 hours at about 24° C and under ordinary room illumination. The purpose of the cut root method was to ensure rapid uptake of the mutagenic solution, and subsequent transport through vascular tissue to the apical stem meristem of the young plant.

3) Injection. Injections of 5 to 10 ml of 0.005 to 0.05 M EMS solutions were made into the lower part of the tassel of young plants with a hypodermic needle and syringe. Test injections with dyes indicated an even distribution of the solution among florets. A few mutants were obtained with EMS by this method; however, treatment of the seed or very young seedlings was found to be more convenient and effective for the purposes of these experiments.

For radiation treatments seeds were exposed to x-rays after the same presoaking procedure as described above for EMS; dry seeds were irradiated with reactor-generated fast neutrons. The x-ray treatments were made with a G. E. Maxitron apparatus operated at 250 kVp, 30 ma, 1.0 mm Al filter, and 30 cm target distance. The soaked seeds were kept moist in a petri dish, placed on a turntable and exposed to 1 and 2 kr of x-rays at a dose rate of 1272 r/min. Fast neutron irradiations were carried out in the thermal column of the Brookhaven Graphite Reactor by using a U-235 converter plate. Dosages of 1 and 2 krads were delivered to dry seeds at a rate of 138.5 rads/min.

The results of treatments with EMS and with radiation, as observed in F_1 kernels, are summarized in Table 1. The data are grouped according to whether the treatment was applied early, i.e., to seeds or young seedlings; or late, i.e., applied to the tassel or pollen. If applications were made at an early stage of embryo or plant development, most mutations appeared in large sectors. This was shown by: 1)the appearance of large chlorophyll deficient sectors in treated plants, particularly in $\underline{Yg_2}/\underline{Yg_2}$ heterozygotes; 2) in female reproductive tissue by the appearance of areas of mutant kernels on ears of treated plants pollinated with a recessive tester; and 3) in male reproductive tissue, by large chlorophyll deficient sectors in the tassel of treated plants as well as by a high frequency of mutant endosperm kernels of the same type on ears of recessive tester plants fertilized with

pollen from the tassel of treated plants. Therefore, the ear or tassel as a whole was taken as the unit for scoring frequency of mutations produced; each cluster of the same mutation was equated to a single mutation. On the other hand, when treatments were made at later stages, by injection of EMS into young tassels or by x irradiation of mature pollen, each single mutated kernel among the total number of kernels scored was considered to be an independently induced mutation.

Table 1
Mutations Induced by Ethyl Methanesulfonate and Radiation in Maize

Mutagen	Method or material	Total number of:	No. mutations			
			Single			Multiple
			C	sh	wx	
Seed or seedling treatment						
		Ears &/or tassels				
Fast neutrons	dry seed	193	3	1	1	0
X ray	wet seed	228	0	0	0	0
EMS	soaking	1158	18	20	24	1*
	root cut	149	3	5	2	0
Control	soaking	502	1†	0	0	0
	root cut	58	0	0	0	0
Tassel or pollen treatment						
		Kernels				
X ray	pollen	1004	1	1	1	6
EMS tassel	injection	4994	0	2	3‡	0
Control	pollen	181	0	0	0	0
tassel	injection	471	0	0	0	1§

* sh wx, but only wx appeared in next generation.

† The plant grown from this was weak and produced no seed.

‡ Two of these kernels came from the same tassel treated.

§ C sh wx, but only wx appeared in next generation.

The combined scoring of early EMS treatments yielded 5.4% (62 out of 1158) single mutations from soaked seed and 6.7% (10 out of 149) by the cut root method. A few cases of exceptionally high incidence of single locus mutations from early treatments were observed. In one, 4 out of 16 ears, and in another 8 out of 27 ears, had a mutation in one of the three loci scored. One multiple locus mutation (sh wx) was obtained from EMS treatment and, in the progeny test, only the wx mutation was transmitted. The high incidence of single locus mutations from early EMS treatments may be due in part to consequences of severe screening of large chromosomal deletions during development of the plant tissue (diploidal elimination) or in reproductive stages (haploidal elimination). However, no multiple locus mutations were obtained from tassel injections with EMS.

The mutations listed in Table 1 were all induced in 1963 and a representative sample (1 to 5) of mutant kernels, with the exception of those induced by x-ray treatment of pollen, were sown in 1964 to confirm the mutations by testing their transmission (Table 2). Of the 24 colored (non-I) mutations from treated material, all germinated and 16 or 17 were confirmed as c-type mutations. Of the 28 sh mutations tested, 24 germinated and 19 were confirmed as transmissible. One of those not transmitted was from a tassel injection treatment and may have been due to noncorrespondence between two generative nuclei that divided prior to the mutation event. Of the 31 wx mutations tested, all germinated, and all but two were transmitted to the next generation. One of these was induced by fast neutrons and was accompanied by loss of all other markers on half the kernel. The other was from late EMS injection and may have been due to noncorrespondence of mutation in the two generative nuclei as noted above.

Pollen fertility in the F_1 of the 29 transmissible wx mutations induced by EMS was checked under low magnification after staining with I_2-KI. More than 90% fertility was found in 22 of these wx mutants. Of the 18 sh mutations produced by EMS treatment 17 were tested for pollen fertility and 16 were found to be more than 90% fertile. Of the 14 definitely confirmed c mutations produced by EMS, all were tested and 13 showed more than 90% pollen fertility. In the I Wx→c Wx mutant, which was of low fertility in the F_1 with the C sh wx tester, the cause of pollen abortion appeared to be independent of chromosome 9 since the ratio of Wx (black) to wx (brown) grains was approximately normal.

Table 2
Progeny Test Confirmation of Induced Mutations

Treatment	No. mutations observed	No. tested mutations germinated	No. mutations confirmed
wx mutations			
Fast Neutrons	1	1	0
Early EMS appl.	27	27	27
EMS injection	3	3	2
sh mutations			
Fast neutrons	1	1	1
Early EMS appl.	25	21	17
EMS injection	2	2	1
c mutations			
Fast neutrons	3	3	2
Early EMS appl.	21	21	14(15)
EMS injection	0	-	-

The interpretation that true gene mutations, free of major change in chromosome structure, may be produced in Zea mays by EMS is encouraged by: 1) induction almost exclusively of single locus mutations; 2) the high fertility of most mutants and normal segregation of chromosome 9 markers in some; 3) alternative explanations to deletion for the I→c mutations; and 4) preliminary evidence of recombination among induced wx mutants.

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2. Mechanisms of genetic recombination.

In 1928 Stadler irradiated maize plants in meiosis and reported no significant effect on intergenic recombinations. Since O. E. Nelson has demonstrated intragenic recombination at the waxy locus in maize an opportunity is provided to determine the effects of irradiation on intragenic recombination.