With respect to the relationship between an enhancement of recombination frequency and stage of meiosis it was found that: (1) the heteroallelic cross wx^{C} x wx^{90}-1 gave a significant and higher increase in recombination frequency, and had a higher proportion of cells in pachynema (Table 2) than wx^{C} x wx^{90}-2. (2) In the heteroallelic cross wx^{C} x wx^{H21} the same relationship was found between recombination frequency and stage of meiosis. However, the increases in recombination were not significant and the proportion of stages in pachynema was lower than in either of the wx^{C} x wx^{90} crosses. (3) In the cross wx^{90} x wx^{H21} the relationship between recombination frequency and proportion of cells in pachynema is evidently not the same as above in that, although both plants gave a significant increase in recombination, the plant with the lower proportion of cells in pachynema gave the higher recombination frequency.

Table 2
Stages of Meiosis (Percentage) of Irradiated Heteroallelic Crosses

	Pre-pachytene	Pachytene	Diplotene	Diakinesis	Metaphase	Anaphase	Telophase
wx^{C} x wx^{90}-1	30.8	69.2	--	--	--	--	--
wx^{C} x wx^{90}-2	--	26.9	59.8	13.3	--	--	--
wx^{C} x wx^{H21}-1	--	--	--	--	72.3	16.1	11.6
wx^{C} x wx^{H21}-2	--	19.0	70.3	5.5	3.2	2.0	--
wx^{90} x wx^{H21}-1	21.3	39.9	23.9	14.9	--	--	--
wx^{90} x wx^{H21}-2	--	2.8	54.2	38.5	4.5	--	--

The wx^{90} and wx^{H21} alleles lie closest together. It is with the heteroallelic cross between these alleles that irradiation produced the greatest increase in genetic recombination.

There is some indication from these studies that irradiation in prepachynema and pachynema may be critical stages for stimulating recombination. However, it is realized that maize may not be the most desirable organism to determine this.

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9. Experiments with ethyl methane sulfonate (EMS) and radiation.

Two experiments have been conducted to study the mechanism of mutation induction by EMS.

I. The possibility of point mutation or inactivation of a small segment of chromosome was tested using four endosperm markers located on the short arm of chromosome 9. Seeds, homozygous for I Sh Bz Wx, were soaked in 0.1 M or 0.05 M aqueous solution of EMS at 25° C for 2 or 5 hours, without buffer, following 24 hours of presoaking in 25° C running tap water. The plants from the treated seeds showed markedly reduced growth, but survived well in the field. They were used as male and as female parents with the recessive tester, C sh bz wx/C sh bz wx. The results are summarized in Table 1. As the plants from the treated seeds showed

Table 1

Loss of Expression of Endosperm Dominant Markers. I Sh Bz Wx Homozygous Seeds Were Presoaked in Water, Soaked in EMS Solution and Crossed with the Recessive C sh bz wx Homozygous Tester. Culture 627 Is the Tester Stock and Those with the Letter E Are the Treated Stocks.

Female	Male	Treatment	No. tested		No. of mutations	Kind of mutation	No. of seeds mutated
			plants	cobs			
627	62E1	Control	27	40	0	-	0
62E1	627	Control	91	119	0	-	0
627	62E2,9,10	EMS soaking*	163	163	2	wx;sh	4 wx; 2 sh
62E2,9,10	627	EMS soaking*	238	242	2	wx;wx	31 wx;13 wx

* 62E2 = 0.1 M EMS for 2 hours, responsible for wx;sh mutations.

62E9 = 0.05 M EMS for 2 hours, responsible for wx;wx mutations.

62E10 = 0.05 M EMS for 5 1/4 hours, no mutations found.

chimeric mutated or altered tissue, in both tassels and ears, the following remarks may help in evaluating the mutagenic effect of EMS. When the treated plants were used as males, pollen was collected from the whole tassel of each plant, and sometimes pollinated to a few tester ears, so the number of plants tested should be emphasized rather than the number of cobs or seeds. On the other hand, when the treated plants were used as females, each cob may be regarded as an independent unit, even though more than one was obtained from a single plant. Table 1 shows that four independent mutations or losses of dominant markers were obtained, three wx and one sh. In all four cases, the possibility of the deletion including bz, which is located between sh and wx, cannot be excluded because of a color inhibitor I, but the losses of expression of only one proximal marker, Wx or Sh, may be noteworthy.

EMS was also applied to seedlings as drops in the opening of the young leaves split vertically with a razor blade, but this method was not as successful as the soaking method.

The nucleoside analogue, 5-bromodeoxyuridine, with or without 5-fluorodeoxyuridine, was also applied by soaking seeds, but at the concentration of 5×10^{-4} M BUdR, neither reduction of growth nor mutation was seen.

II. Somatic mutation in Yg_2/yg_2 heterozygotes was tested to determine the dose-response relationship of mutagenic agents. This system, in which losses of the dominant green marker (Yg_2) are expressed as small yellow-green sectors in leaves, worked well with lower dosages of the mutagen tested. The dosage which gave about 20% reduction of seedling height induced maximum numbers of sectors for reliable scoring. For scoring purposes both leaf 3 and leaf 4 of greenhouse-grown plants were used, but only the data from leaf 3, which gave more sectors than leaf 4, will be reported here.

A. X rays. Four combinations of Yg_2 and yg_2 were tested for response to X-irradiation after 24 hours presoaking in running tap water at 25° C. Curves were constructed from the data given in Table 2. Yg_2 homozygote showed a two-hit type of response (number of sectors increased quadratically with dose). This is as expected because there are two alleles of Yg_2 or of other dominant genes governing normal green chlorophyll production. The two reciprocals of heterozygotes showed a very rapid increase of sector frequencies with an increase in X-ray dose. Differences observed between the reciprocals might be due to a difference in hydration during presoaking. The response to increased radiation dose of these two heterozygotes seemed to be more linear than quadratic. The recessive yg_2 homozygote also showed yellowish sectors (lighter than the background tissue), but the yield of sectors was insufficient to interpret in terms of the type of response curve produced.

B. Fast neutrons. As a type of densely ionizing radiation, fast neutron irradiation of dry seeds of the four different stocks was tried, but most of the doses used were too high for reliable scoring of sector frequency. With the lowest dose, 500 rads, the frequency of yellow-green sectors was highest in both heterozygotes. Yg_2 homozygotes showed fewer sectors and the yg_2 homozygote showed least sectors among the four stocks.

Table 3

Frequency of Yellow-green Sectors in Leaf 3. Seeds Were Presoaked in Water for 24 Hours at 27° C and Soaked in EMS solution for 5 Hours at 27° C. Fifteen Seeds Were Used in Each Lot.

EMS Concentration (M/l)		0	0.005	0.01	0.02	0.03	0.04	0.05	0.06
$Yg_2,Y/yg_2,y$	1.	0	2.067	6.333	11.000	11.643	17.000	(13.00)	(15.50)
$Yg_2,Y/yg_2,y$	2.	0.071	1.786	5.400	10.231	11.154	(19.000)	(6.00)	
$Yg_2,Y/Yg_2,Y$	1.	0	0.533	1.539	2.667	5.083	(5.625)		
$Yg_2,Y/Yg_2,Y$	2.	0.067	1.071	1.733	2.167	6.000	(6.000)		

( ): Less than 70% of leaves scorable.

Table 2
Frequency of Yellow-Green Sectors in Leaf 3*

Genotype	X-ray dose, r						
	0	250	500	750	1,000	1,500	2,000
Yg_2/Yg_2	0.00	0.032	0.158	0.444	1.200	(1.692)	(1.214)
Yg_2/yg_2	0.050	1.258	2.211	1.722	(1.667)	(1.875)	(1.000)
yg_2/Yg_2	0.050	0.419	0.850	1.631	2.313	2.882	2.400
yg_2/yg_2	0.00	0.097	0.100	0.111	0.250	0.444	0.133
No. of seed in each lot	20	32	20	20	20	20	20

*Seeds were presoaked in running tap water at 25° C for 24 hours, then X-rayed.
( ): Less than 70% of leaves scorable.

C. EMS. In this experiment, only Yg_2, Y/Yg_2, Y and Yg_2, Y/yg_2, y were used. These two stocks of seeds were very alike in appearance, except that the color of endosperm was slightly lighter yellow in the heterozygotes (YYy) than in the homozygote (YYY). Seeds were soaked in 0.005 M to 0.06 M fresh EMS solutions for five hours at 27° C after 24 hours of presoaking in oxygenated deionized water at 27° C. Results are shown in Table 3. The yield of yellow-green sectors in the heterozygotes was about twice that in the homozygous Yg_2, and 5 to 7 times higher than in the X-ray treatment at their maximum yields. With EMS, however, the response of yield relative to the concentration was approximately linear in both stocks. Although many variables accompanied by soaking treatment must be investigated for proper interpretation, the result is interesting for the study of the mutation-inducing mechanisms of EMS.

D. Temperature and EMS. The effect of temperature during post incubation of EMS-treated seeds was tested with a yg_2 heterozygote. Seeds were soaked in 0.025 M EMS solution for 48 hours at 3° C, after presoaking as described above in the X-ray experiment. Seeds were rinsed with cold (3° C) water and incubated in the dark at several different temperatures ranging from 3° C to 33° C. Yield of the sectors increased at the higher temperatures tested, up to about twice as much as at the low temperature.

E. Recent mutations with EMS. A partial survey of the 1963 harvest from EMS-treated material has revealed 4 mutations in chromosome 9 marker genes. The treatments consisted in soaking seeds of a homozygous dominant stock for 24 hours at 27° C in water, then in 0.05 M EMS (5 hours at 27° C or 48 hours at 3° C). The silks of plants developed from EMS-treated seeds were pollinated with a multiple recessive stock. Four independent mutations were found on 4 different ears in a total of 181 cobs scored to date. These mutations were 1C, 1sh, and 2wx.

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