

The muons were found to have about the same mutational efficiency as the X rays (average RBE = 1.01). The calculated results for π^- mesons indicated a relative mutational efficiency of about 3 (average RBE = 3.16). The greater effectiveness of π^- mesons is considered to be due to the energy deposition through strong interactions of these particles with protons and neutrons of the atomic nuclei. These interactions result in nuclear disintegrations producing stars and showers of lightly ionizing and heavily ionizing secondary particles such as protons, alpha particles and heavier nuclei. Since these higher LET tracks are much more efficient than X ray or other low LET radiations (such as muons) in breaking maize chromosomes, π^- mesons would be expected to have the higher RBE observed.

A. Micke
H. H. Smith
R. G. Woodley
A. Maschke

8. Effects of X-irradiation on intracistron recombination at the Wx/wx locus.

Research work by O. E. Nelson (Science 130:794) has demonstrated intracistron recombination at the waxy locus in maize. Also, Roman (CSHSQB, 1958) has demonstrated an increased rate of intragenic recombination in yeast by using ultraviolet irradiation. This research was conducted to determine if X-irradiation would influence the rate of intracistron recombination in the waxy locus in maize.

The present authors are grateful to Dr. Oliver E. Nelson for providing the seed stocks described in this research. The procedures used for the pollen assay are essentially those published by Nelson.

Waxy alleles, wx^C , wx^{90} and wx^{H21} were available in the homozygous condition and all possible combinations among the parents. The wx^C and wx^{H21} stocks had been backcrossed six times, and wx^{90} three times, to inbred M14.

An acute dose of 200 r of X-irradiation was applied to each maize plant during meiosis. The plants were irradiated in air with a G.E. Maxitron 250 X-ray machine (30 ma, 250 kv, 1 mm Al filter at 50 cm).

At the time of irradiation sporocytes were collected. These were later scored as to the stage of meiosis. The area of collection was marked with ink in an attempt to simulate a "synchronous" system and to determine the stage(s) of meiosis at which the tassel was irradiated. The marked tassel area was used as the center of the target area and pollen used in the assay was taken from this area. Tassels to be used in the pollen assay were collected before anthesis and stored in 70% alcohol.

Nelson (1959) states that if the frequency of black staining (Wx) pollen grains in the population from a cross between two mutants is significantly higher than the frequency in either parental stock, this would indicate that the two mutations occupy different sites within the region.

The figure for each stock presumably could include back mutation, suppressor mutation and contamination from wind-blown pollen.

In our research we used a similar approach. Parents and the heteroallelic crosses were irradiated and in addition, unirradiated parents and heteroallelic crosses were used as controls. Half the \underline{Wx} frequency of each control parent (spontaneous back mutation, etc.) was subtracted from the \underline{Wx} frequency of the control heteroallelic material. Also half the \underline{Wx} frequency of each irradiated parent (spontaneous and induced back mutation, etc.) was subtracted from the \underline{Wx} frequency of the irradiated heteroallelic material. Then the corrected \underline{Wx} frequency of the control heteroallelic material was compared with the corrected \underline{Wx} frequency of the irradiated heteroallelic material (Z test).

The data in Table 1, for the irradiated and control parents and control heteroallelic crosses, are bulked from several plants. However, the data for each irradiated heteroallelic cross are from one plant.

Our results, Table 1, with the control parents and the heteroallelic material gave a lower rate of back mutation and of spontaneous recombination rate than reported by Nelson (1959). In his research, Nelson points out that the residual genotype may affect the recombinational process within the waxy locus, and this may apply here.

Some researchers assume that genetic recombination takes place in zygonema and pachynema, i.e. when the chromosomes are paired. Pontecorvo (1958) states that crossing over may take place well before meiotic prophase. It may be assumed that the stage in which recombination takes place is the critical stage to affect recombination, but this may not necessarily be so. In an attempt to elucidate this problem the stages of meiosis at the time of irradiation were recorded.

Irradiation apparently increased the rate of recombination in each treated heteroallelic cross, except one (Table 1); however, statistical significance was obtained for only three of them.

Table 1
Parents and Heteroallelic Crosses

	Control		Irradiated	
	Est. no. of microspores $\times 10^3$	\bar{X} no. $Wx \times 10^{-5}$ $\pm s\bar{x}$	Est. no. of microspores $\times 10^3$	\bar{X} no. $Wx \times 10^{-5}$ $\pm s\bar{x}$
wxC	428	0.00 \pm 0.00	125	0.80 \pm 0.80
wx90	732	0.27 \pm 0.19	530	1.70 \pm 0.57
wxH21	410	0.24 \pm 0.24	92	3.26 \pm 1.88
wxC x wx ⁹⁰ ₋₁	644	28.40 \pm 2.10	82	72.67 \pm 9.38*
wxC x wx ⁹⁰ ₋₂	644	28.40 \pm 2.10	168	18.48 \pm 3.32
wxC x wxH21 ₋₁	335	27.77 \pm 2.88	99	33.27 \pm 5.79
wxC x wxH21 ₋₂	335	27.77 \pm 2.88	50	41.81 \pm 9.12
wx90 x wxH21 ₋₁	235	1.28 \pm 0.74	160	13.14 \pm 2.87*
wx90 x wxH21 ₋₂	235	1.28 \pm 0.74	97	22.57 \pm 4.81*

* Exceeds 1% level of significance.

With respect to the relationship between an enhancement of recombination frequency and stage of meiosis it was found that: (1) the heteroallelic cross $wx^C \times wx^{90-1}$ gave a significant and higher increase in recombination frequency, and had a higher proportion of cells in pachynema (Table 2) than $wx^C \times wx^{90-2}$. (2) In the heteroallelic cross $wx^C \times wx^{H21}$ 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 $wx^C \times wx^{90}$ crosses. (3) In the cross $wx^{90} \times wx^{H21}$ 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	Diplo- tene	Dia- kinesis	Meta- phase	Ana- phase	Telo- phase
$wx^C \times wx^{90-1}$	30.8	69.2	--	--	--	--	--
$wx^C \times wx^{90-2}$	--	26.9	59.8	13.3	--	--	--
$wx^C \times wx^{H21-1}$	--	--	--	--	72.3	16.1	11.6
$wx^C \times wx^{H21-2}$	--	19.0	70.3	5.5	3.2	2.0	--
$wx^{90} \times wx^{H21-1}$	21.3	39.9	23.9	14.9	--	--	--
$wx^{90} \times wx^{H21-2}$	--	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 pre-pachynema 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.

R. W. Briggs
H. H. Smith

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