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MASTER

NINE SHORT ARTICLES

on Studies of Zea mays

From the Laboratory of H. H. Smith

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1. Progress towards perennial Zea diploids.

Experiments were reported last year which show that one can make steady progress in increasing the perennial expression of maize-teosinte derivatives at the $4N$ level by the simple breeding technique of repeated mass selection for perennialism. Thus at the 50% maize level, one has been able to increase the incidence of perennial segregates from an original level of about 0.3 to 0.75 in only 4 cycles of selection. Similarly, the production of basal branches, an attribute of perennialism, was increased from about 4.4 per plant to about 10.0. At the 75% maize level, only two generations of selection have increased the incidence of perennial segregates from 0.0 to about 0.35. Unhappily, either genetic or agronomic investigations of perennialism are difficult, if not meaningless, at the $4N$ level.

The situation is entirely different when working at the diploid level. As outlined, one can easily obtain large populations of maize-perennial teosinte diploids by producing the F_1 triploid generation, backcrossing this triploid to maize, and then intercrossing the resultant array of aneuploids. The chromosome number is rapidly stabilized at 20 because of gametophyte selection for euploidy. Due to the high degree of preferential pairing in the F_1 triploid, the first post triploid generation carries a high theoretical proportion of perennial chromatin, somewhere between 40

* Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.

and 45%, depending upon certain assumptions.

Since 4 separate experiments, involving now thousands of putatively perennial segregates derived by as many as 5 successive generations of intensive selection for perennialism or perennial attributes, have completely failed to yield any perennial diploid segregates at all, it is clear that diploidy^{im,} poses special difficulties upon the task of reconstructing a genotype which might confer perennialism. One does not even make progress in increasing the expression of perennial attributes, such as basal branch production. Thus in the 2nd post triploid generation, the number of basal branches per plant was 3.3. In the 3rd generation it was 2.9, and in the 4th, 3.1. At the same time production of apparently normal pollen by diploids increased from 88% to 95%, while the net viability of seeds and seedlings increased from 66% to 85%. (The pollen studies noted here represent scores taken from side-by-side outdoor-grown samples. The studies reported last year [MNL 37: 8-11] were from greenhouse collections, and were extremely erratic by comparison. It is suggested that the data from outdoor collections should be regarded as valid.) These disappointing experiences with diploids can be explained if perennial teosinte, during its evolution as a tetraploid, has accumulated many mutations, such that if one attempts to rediploidize segments carrying them, an essential function may not be represented, and such "sheltered" tetraploid factors then act as haploid gametophyte or diploid sporophyte lethals. If the loci from perennial teosinte which confer perennialism are linked with such lethals, they will be eliminated inexorably in diploids. Moreover, while the phenotype of the triploid F_1 , and to a lesser extent, the first post triploid generation, look decidedly intermediate among maize and teosinte, the

advanced post triploid generations become, in spite of contrarywise selection pressure, extremely maize-like.

One has been able to rule out the idea that one fails to recover perennialism in diploids because of the absence of tetrasomy by directly doubling with colchicine several of these advanced generation diploids. Thus of nine confirmed and 12 probably newly redoubled tetraploids, none showed the slightest degree of perennialism or increase in the expression of perennial attributes, and all died at the same time as their nondoubled sib mates.

Although an exhaustive study has not yet been made, several diploids having perennial teosinte segments have been examined at pachynema. Since no large, classical rearrangements have been found so far, it seems certain that if they exist, they are certainly not generalized, and cannot explain the general elimination of teosinte characters. The weight of evidence is therefore that one has to deal with subvisible, or "genetic" factors.

As reported last year, only one breakthrough in this otherwise bleak picture has been found. One plant, a trisomic, found in a first post triploid generation, "Clone 85-12," was obtained which although very weak and extremely difficult to maintain, did appear to be perennial. Although it is so far completely self sterile, it does produce pollen about 5% of which stains with IKI. Some of this pollen was used to effect fertilization of perennial teosinte ovules. Of the triploids thus obtained, one was fertilized again by 85-12 pollen. Of the resulting aneuploids, two were saved, with 22 and 24 chromosomes respectively. These were grown in greenhouse culture, and upon the demise of the first culms, basal and axillary

began to produce offsets which could be separated and thence develop into fully normal plants which again produced many fully totipotent basal branches. It is sufficient to say that the 22 chromosome derivative, which has been retained as "Clone A," has a vigorous, fully perennial phenotype and ^{it} can be cloned with ease to any desired number of propagules. Out-of-doors it is extremely vigorous, and its basal branches become at least pseudo-rhizomatous. Although it produces about 70% staining pollen, and a multitude of vigorous, 4-rowed distichous ears, it is also nearly self sterile. Only 16 seeds have been obtained from numerous pollinations. Of these, 10 germinated successfully. Two proved to be albinos, while the remaining eight grew to maturity. Three were trisomics, and five were euploids. Even though five of the eight were grown out-of-doors, none showed any perennial tendency or attribute. No basal branches at all were produced under the same conditions in which the sexual parent, Clone A, formed as many as 16 basal branches during one growing season and was still growing strongly when freezing temperatures brought the season to a close. The one ear produced by the sexual derivatives of Clone A was polystichous and the plants were otherwise maize-like. This appears to be the best evidence of all that loci from perennial teosinte are selectively not transmitted in diploids.

While Clone A and 85-12 are nearly self sterile, they may be crossed together, and viable progeny obtained. Of 18 seeds so obtained, 10 grew successfully, one of these appears to be a perennial whose root tips yield perfectly repeatable counts of both 20 and 21 chromosomes.

Clone A may also be crossed with perennial teosinte, and the resulting triploids crossed back to Clone A. From the ensuing aneuploids, one can select those with the lower chromosome numbers for further work.

Unlike previous experiences, all such populations at the near-diploid level which were derived ultimately through 85-12 (by way of Clone A) are essentially perennial populations with a few exceptional annuals. Even though as a group these also tend to be strongly self sterile, a few seeds can be obtained by sib pollination.

In summary, the study of pollen abortion, seedling and seed lethality, and inability to transmit teosinte characteristics, including perennialism, at the diploid level or near diploid level, support the idea that perennialism-conferring loci in teosinte are linked with diploid-lethal factors which make perennialism difficult to recover in diploids. Clone 85-12 may have arisen by means of a fortuitous crossover such that a key perennialism locus may be brought at least to a diploid or near-diploid background. The evidence that 85-12 may represent such a specific event is that besides the fact that near diploids derived through it are not only unique in being essentially all perennial, but all possess, in addition, a photoperiodic response such that on long days only terminal inflorescences are produced, while in a short day regime, both terminal and lateral inflorescences appear. In either regime, however, at least Clone A continues to produce totipotent basal branches, and therefore is still perennial even in a short day regime.

Because of the perennial nature of the material, Clone A now represents an easy means to produce any number of additional perennial diploids and near diploids that could be desired. If the remaining task is essentially one of obtaining fortuitous crossovers in order to place perennialism-conferring loci into maize chromosomes, one then appears to

to be in the position of having the means by which any degree of pressure may be applied to the problem that it may require.

D. L. Shaver

2. A simple mechanical method of inducing tetraploidy.

Heat shock and colchicine treatments, historically speaking, have yielded a very low percentage of success in producing maize tetraploids. Recently, genetic methods of introducing new chromosomes from diploids into existing tetraploids have been proposed and come into vogue. These methods, involving the genes elongate, asynaptic, and ameiotic, however, require several generations of work to bring the inducing gene into the background one wishes to tetraploidize before one succeeds in deriving the desired diploid gametes. In an agronomic situation, one eventually faces the problem as to whether the inducing genes are linked to unfavorable factors. Moreover, the inducing gene itself is introduced into the new tetraploid, where it is then undesirable.

Two experiments have shown that one can easily introduce desired chromosomes from diploids into existing tetraploids by the straightforward procedure of crossing a $4N$ female by the desired diploid. If the resulting shriveled seeds are dissected and the embryo proper is removed and shallowly planted in moist soil, or other situation where the developing embryo can soon persist on its own photosynthate, no difficulty is encountered in growing these triploid embryos. (The dissection procedure was suggested by Ellen Dempsey.)

Triploids produce much pollen in the greenhouse, or out-of-doors on Long Island, but they may not if grown outside in less favorable environments.

One can collect this triploid pollen in conventional pollen bags, or by shaking it directly off of the tassels, and then straining it through a stack of U. S. Standard Sieves (W. S. Tyler Co., Cleveland 14, Ohio). A top screen with a mesh size of 149 microns removes clumped pollen and loose anthers. The next screen with a mesh size of 125 microns catches a small proportion of the very largest pollen grains from triploids (and tetraploids). No diploid has yet been found to produce pollen grains which "stay" in this screen. The next screen with 105 micron mesh catches most of the large grains. These were discarded in this experiment. The next screen with 74 micron mesh catches most of the viable small grains, while the last, 53 micron mesh, seems to catch only aborted or dried grains.

The following results were obtained by sibbing a population of triploids with the 74 and 125 pollen fractions:

74 Micron Pollen		125 Micron Pollen	
Chrom. No.	No. of Individuals	Chrom. No.	No. of Individuals
20	1	23	1
21	1	30	2
22	1	33	3
23	4	34	2
24	2	36	5
25	5	37	4
26	2	38	4
27	1	39	1
28	1		
29	4		
30	1		
32	1		

It is evident that the screening procedure results in two nearly

discrete populations. If one sibs among the 36-39 chromosome individuals, one obtains a new population having many individuals with 40 or more chromosomes. The chromatin of such newly constituted tetraploids should derive approximately 45% from the original diploid parent.

D. L. Shaver

3. A successful colchicine method for Zea.

In producing tetraploids of Zea, the "target" for colchicine action is the undifferentiated meristem of the shoot apex. Probably because of complications involved in the various techniques of getting colchicine through the several multicell-layered embryonic leaves which ensheath the apex, production of tetraploids by means of this mitosis inhibitor has with few exceptions been disappointingly difficult.

Faced with the necessity of deriving tetraploids from a 2N population directly, one used a method of applying colchicine proposed by Dr. L. F. Randolph in one of the early Maize Genetics Cooperation News Letters, in conjunction with colchicine techniques suggested by Dr. J. Van't Hof at Brookhaven. Seeds of maize were germinated until the primary root reached about $1\frac{1}{2}$ " in length. About $\frac{1}{4}$ to $\frac{1}{2}$ inch of the root tip was cut off under distilled water to expose the large, empty xylem vessels of the differentiated portion of the root. The seedling was then suspended on hardware cloth above an aqueous colchicine solution in such a way that only the decapitated root tip was immersed, and the young seedling therefore was forced to supply its entire transpiration stream through the decapitated root.

Effective colchicine concentrations were determined by dissecting

out the shoot apex, smearing, and determining the proportion of colchicine metaphases present. In this manner, proper concentrations were determined for the specific material used. Possibly another strain or variety could have a different optimal level. In this work, one chose the lowest level at which all observed metaphases were "arrested." It was found that there is an accumulation of colchicine effects from one treatment period to the next. Thus, 0.025% colchicine did not produce 100% arrested metaphases during the first 24 hr treatment, but during the second 24 hr treatment, all were arrested. The practice was eventually adopted of using ~~a~~ higher concentration for the first cycle of treatment than during succeeding ones.

In the first experiment, 500 maize seedlings were given three 24 hr treatments in 0.05% colchicine, interrupted by 24 hr recovery periods in distilled water. Of the 16 survivors of this treatment, 4 were confirmed tetraploids (their pollen produced full seed sets when tested upon an established tetraploid), and 2 were probably tetraploid (produced pollen, some of which "stayed" on a 125 micron screen). In the second experiment, 500 maize seedlings were treated for the first 24 hours in 0.05% colchicine, allowed to recover for 24 hours, and then treated for an additional 24 hours with 0.025% colchicine. Of 152 survivors of this treatment, 5 were confirmed tetraploids and 10 were probables. There appeared to be only one case of 2N-4N sectoring. The rest appeared to be cases where the entire shoot apex had become tetraploidized.

Dr. J. Van' Hof's "colchicine bible": Use colchicine from a source where the quality of the chemical is consistent, e.g. G. T. Gurr Ltd., London, S.W. 6, England, and store in the dark in a desiccator over CaCl_2 . Always make up fresh solutions immediately before use. Always establish optimal

levels for each new variety by experimental dissections and fixations.

D. L. Shaver

4. A mechanical method of classifying large number of seeds for waxy.

The work of O. E. Nelson of establishing the map characteristics of the wx cistron of maize has established an exceedingly useful system for the study of the nature of mutation in a higher organism. However, in the initial steps of producing and screening for mutations produced by radiations or chemical mutagens, one is faced with a Herculean task of physically classifying maize kernels for wx in sufficient number that one can realistically expect to recover a meaningful number of induced mutants.

In order to speed this task, a Red Devil paint shaker model 30 was obtained. The sides of two one-gallon paint cans were lined with floor sanding paper with a no. 2 grit (Behr-Manning Co., Troy, N.Y.). The machine was connected to a standard greenhouse timing switch so that runs could be made while the machine was unattended. It was found that this machine could easily scarify 2000 grams or ca. 11,000 kernels at one time. Doubtless it could be adapted to larger containers and this could be greatly increased. It was necessary to liberally perforate the ends and sides of the cans with small holes to allow the dust to sift out, and ^{thus} avoid "filling" of the sandpaper. Frictional heating of the can and its contents could be avoided by setting the timing switch to 15 minutes on, 15 minutes off, etc. Two and one half to three hours running time was sufficient to scarify the seeds for wx classification.

As a result of this scarification treatment, the pericarp and aleurone layers were uniformly worn away, especially on the edges and tips

of the kernels, while a recessed "germ" or embryo usually escaped damage. Such seeds could then be dipped in weak IKI solution, and rapidly spread upon an absorbent material to dry while being classified for wx. Searching for mutant kernels was as simple as looking for a red-brown ball among many black ones. Small batches were stained at a time, after rinsing away the loose dust, and immediately searched for mutants since the contrast between wx and Wx kernels is best just after staining.

It should be emphasized that the usefulness of the method depends upon having kernels to work with in which the germ is recessed. This was the case in well-pollinated ears of M14, but is not true of many other inbred lines. From a practical point of view, even with M14 the method will be found unsatisfactory unless really full sets of seed are obtained so that resulting kernels will be flat instead of round. Perhaps isolation-detasseling production of subject kernels is the most practical way of obtaining the needed numbers and quality of seed.

D. L. Shaver

5. id maize.

Several attempts have been made to mate id/id plants carrying a newly found id gene (Shaver, MNL 31: 94) with id/id plants having the classical C30 id allele (Galinat and Naylor, AJB 38: 38-47). Various manipulations of photoperiod have succeeded in inducing flowering, but the small ears produced have always been barren. Since it seemed impossible to mate homozygous id plants, an alternative procedure was employed, that of mating normal plants in selfed, segregating families between the two id sources. Of 16 ear progenies so obtained and grown in Florida this winter, 7 segregated for

the id/id phenotype, indicating that the id genes from the two sources are allelic. It is interesting that the id/id plants, planted November 10, 1963, were not induced as of January 28, 1964 in spite of the fact that they were grown in a regime which induces teosinte, since for example, the interval between sunrise and sunset on December 21 is only 10½ hours. This experience agrees with observations in Florida a year ago. Homozygous id/id plants, seeded October 15, 1962 were not induced as of March 10, 1963 at Princeton, Florida.

D. L. Shaver

6. Relative biological efficiency of monoenergetic fast neutrons on chromosomes in maize.

Investigations on the relative biological effectiveness (RBE) of densely ionizing radiations (with high LET, rate of linear energy transfer) are of importance in both fundamental and applied radiobiology. The difficulty in determining RBE on the basis of chromosomal exchanges or 2-break aberrations is that the dose-response curves differ for radiations of different LET and dose rate. Maize seeds of Yg₂/yg₂ genotype were used to study the RBE of fast neutrons vs. X rays.

The maize material used in these experiments has the advantage for RBE studies of yielding a basically first order dose-response curve ($Y = \alpha + \beta D$) with low (X rays) as well as with high (fast neutron) LET radiations. The frequency of yellow-green (yg₂) sectors in leaves 3, 4 and 5 of young plants grown from irradiated Yg₂/yg₂ seeds served as a quantitative measure of response. The mutant sectors are believed to be due mostly to simple chromosome breakage and deletion. An exposure apparatus

was used which produced essentially equal dose rates in five rings of seeds placed so as to intercept neutrons of 0.43, 0.65, 1.00, 1.50 and 1.80 MeV. Dose average LET values for these energies are 72, 67, 58, 57.5 and 42.5 kev/ μ , respectively.

Two experiments were performed at dosages that gave responses which were linear, below saturation levels, and overlapping in range for X rays and neutrons. These ranges in dosages were 32.8 to 126.4 rads of neutrons and 1500 to 15,600 rads of 250 kvp X rays.

RBE values, calculated from relative slopes (α) of linear regression lines for N and X, ranged from 42 to 135 (average 78) in Experiment 1 and from 48 to 106 (average 68) in Experiment 2. Monoenergetic fast neutrons of 0.43 MeV were the most efficient in producing yg₂ sectors as shown by the yield of sectors per krad and highest RBE values.

The RBE values obtained in these experiments are higher than commonly reported and in the neighborhood of those found by Neary et al. (Int. J. Rad. Biol. 6: 127) for plant chromosomes when the dose-squared term of low LET radiation response is minimized. With regard to maximum permissible levels of radiation for man, these results suggest the alternatives that either chromosome breaks in plants have a much higher RBE than comparable reactions in man and need not be considered; or that the problem of chromosome damage per se in human tissues be reexamined after exposure to high LET radiations and/or low LET radiations at low doses or dose rates.

H. H. Smith

7. Relative biological efficiency of muons and π^- mesons.

Until recently meson beams of sufficient intensity for cytogenetic

studies have not been available. The Alternating Gradient Synchrotron (AGS) at the Brookhaven National Laboratory now produces almost pure π^- meson and muon (μ^- particle) beams suitable for use in biological experiments. The mesons were generated in the AGS by bombarding a beryllium target with highly accelerated protons of about 28 BeV energy.

Dormant seeds of $\underline{Yg}_2/\underline{yg}_2$ maize were exposed in two experiments:

(1) to 1275 rads of nearly pure muons; and (2) to 3360 rads, comprising about 2060 rads of muons and about 1300 rads of π^- mesons. To compare these effects with those of better known radiations, seeds of the same material were treated with 250 kVp X rays. The frequency of $\underline{yg}_2$ sectors was scored in leaves 3, 4, 5 and 6 of the seedlings.

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

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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 acknowledge 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 Wx frequency of each control parent (spontaneous back mutation, etc.) was subtracted from the Wx frequency of the control heteroallelic material. Also half the Wx frequency of each irradiated parent (spontaneous and induced back mutation, etc.) was subtracted from the Wx frequency of the irradiated heteroallelic material. Then the corrected Wx frequency of the control heteroallelic material was compared with the corrected 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. $Wx \times 10^{-5}$ $\pm s\bar{X}$
wx^C	428	0.00 ± 0.00	125	0.80 ± 0.80
wx^{90}	732	0.27 ± 0.19	530	1.70 ± 0.57
wx^{H21}	410	0.24 ± 0.24	92	3.26 ± 1.88
$wx^C \times wx^{90-1}$	644	28.40 ± 2.10	82	$72.67 \pm 9.38^*$
$wx^C \times wx^{90-2}$	644	28.40 ± 2.10	168	18.48 ± 3.32
$wx^C \times wx^{H21-1}$	335	27.77 ± 2.88	99	33.27 ± 5.79
$wx^C \times wx^{H21-2}$	335	27.77 ± 2.88	50	41.81 ± 9.12
$wx^{90} \times wx^{H21-1}$	235	1.28 ± 0.74	160	$13.14 \pm 2.87^*$
$wx^{90} \times wx^{H21-2}$	235	1.28 ± 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 sulphonate (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 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 see the dose-response relationship of mutagenic agents. This system, in which losses $\leftarrow$ 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.

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 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.

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 5½ hours, no mutations found.

Table 2. Frequency of yellow-green sectors in leaf 3. Seeds were presoaked in running tap water at 25° C for 24 hours, then X-rayed.

Genotype	X-ray dose, r						
	0	250	500	750	1000	1500	2000
<u>Yg₂</u> / <u>Yg₂</u>	0.00	0.032	0.158	0.444	1.200	(1.692)	(1.214)
<u>Yg₂</u> / <u>y_{g2}</u>	0.050	1.258	2.211	1.722	(1.667)	(1.875)	(1.000)
<u>y_{g2}</u> / <u>Yg₂</u>	0.050	0.419	0.850	1.631	2.313	2.882	2.400
<u>y_{g2}</u> / <u>y_{g2}</u>	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

(): Less than 70% of leaves scorable.

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
$\underline{Yg_2}, \underline{Y}/\underline{Yg_2}, \underline{Y}$	1.	0	2.067	6.333	11.000	11.643	17.000	(13.00)	(15.50)
$\underline{Yg_2}, \underline{Y}/\underline{Yg_2}, \underline{Y}$	2.	0.071	1.786	5.400	10.231	11.154	(19.000)	(6.00)	
$\underline{Yg_2}, \underline{Y}/\underline{Yg_2}, \underline{Y}$	1.	0	0.533	1.539	2.667	5.083	(5.625)		
$\underline{Yg_2}, \underline{Y}/\underline{Yg_2}, \underline{Y}$	2.	0.067	1.071	1.733	2.167	6.000	(6.000)		

(): Less than 70% of leaves scorable.

E. Amano

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