THE EFFECTS OF X RAYS ON THE BRONZE AND SHRUNKEN LOCI IN MAIZE[1]

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SINCE the discovery by MULLER with Drosophila (1927) and by STADLER with barley (1928) that the frequency of visible and lethal mutations is greatly increased by X-irradiation, a wide variety of organisms has been subjected to X-ray treatment by different investigators to elucidate the nature of the induced genetic changes. Although it is generally accepted that the majority of these mutations are associated with chromosomal alterations ranging from gross structural changes to minute deletions and inversions, there are discordant results and divergent opinions on whether or not X rays can also effect intragenic changes. The earliest work by PATTERSON and MULLER (1930) and by TIMOFEEFF-RESSOVSKY (1932) indicated that reverse mutations could be induced at selected loci in Drosophila. These induced reversions were assumed to be diagnostic of intragenic mutations. Contrary results, however, were obtained by LEFEVRE (1950) in extensive experiments similar to those mentioned above. Subsequent data (MULLER and OSTER 1957; LEFEVRE and GREEN 1959; GREEN 1961) showed that X ray-induced reversions do occur in Drosophila and that the earlier negative data may be explained in terms of the stages at which cells were treated or by the particular alleles employed.

The evidence from studies with maize is in agreement with that reported by LEFEVRE (1950) in his Drosophila work. In an extensive experiment, STADLER (1944) was unable to induce somatic reversions of the a_1 allele. Other investigations by STADLER and ROMAN (1948) dealt with the production by X rays of direct mutations at the A_1 locus. Among approximately 415 plants which exhibited loss of the A phenotype, three appeared as putative intragenic mutations and were subjected to extensive genetic and cytological tests. All three proved to be minute deficiencies which included the A locus, rather than intragenic alterations.

From other studies (EMMERLING 1955; NUFFER 1957) no unequivocal evidence has been found for the induction by X rays of intragenic mutations in maize.

Unlike the results in maize, evidence in Neurospora indicates that intragenic mutations are produced by X-irradiation (GILES, DE SERRES and PARTRIDGE 1955; DE SERRES 1957). In fact, such alterations have been partially characterized at the nucleotide level (MALLING and DE SERRES 1967).

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In experiments conducted by RUSSELL and RUSSELL (1959, 1960), mice were irradiated and mutations induced at a premeiotic stage of spermatogenesis were analyzed. Results indicated that some of the alterations might be intragenic in nature but the data were inconclusive.

The present investigation is patterned after the studies of STADLER and ROMAN (1948). Since their experiments were confined to a single locus in maize, their negative findings do not warrant a generalization about the inability of ionizing radiations to induce gene mutation in maize. Additionally, the A_1 locus may not be a representative gene and it thus seemed advisable to conduct similar studies on X-ray mutagenesis with another gene in maize.

MATERIALS AND METHODS

A study of the genetic effects of X rays in maize requires a gene with a phenotype readily identifiable at various stages of the life cycle whose mutant form has no adverse effect on the viability of the sporophyte or gametophyte generations. A mutant phenotype which is easily scorable at the seed and seedling stage is especially desirable since large populations are more easily handled and more rapidly classified at those stages. Availability of flanking marker genes to facilitate crossover studies is also a criterion for selection of the locus to be studied. The bronze-1 locus chosen for the present investigation fulfills these requirements.

Bronze-1 is located in the short arm of chromosome *9* at map position 31. Shrunken-1 is approximately two map units distal to bronze, and waxy, about 18 units proximal. The dominant allele of bronze (*Bz*) in the proper genetic background produces a purple color in the plant and kernel while the recessive (*bz*) phenotype is bronze. The phenotypes produced by the dominant and recessive alleles of shrunken and waxy are as follows: *Sh*–plump kernel; *sh*-collapsed kernel; *Wx*–starchy endosperm and pollen (stain blue with potassium iodide); *wx*–waxy endosperm and pollen (stain red with potassium iodide).

The populations to be screened for mutations at the *Bz* locus were produced in two crosses involving *Bz* stocks. In cross #1, mature pollen from plants homozygous for *Sh*, *Bz* and *Wx* was irradiated and placed on silks of plants homozygous for *sh*, *bz* and *wx*. A dosage of approximately 1000r screened by a 3 mm Al filter was applied in five minutes using a GE Maximar 100 machine operating at 100 kv and 5 ma.

In maize, the second microspore division occurs shortly before anthesis. If mature pollen is treated, mutations induced in one sperm nucleus will not be found in the other. Hence the phenotype of the embryo and endosperm of a kernel derived from such a pollen grain will not correspond since a mutant sperm nucleus fertilizes either the polar nuclei or the egg nucleus but not both. Therefore, the F_1 kernels with a *bz* endosperm detected in cross #1 will usually not produce *bz* plants and screening of seedlings for *bz* mutations is necessary.

In an attempt to induce mutations in the generative nucleus before the second microspore division, the male parents in cross #2 were treated approximately 16–19 days prior to anthesis. A dose of approximately 1000r was applied through a 3mm Al filter using a Picker V2 machine operating at 220 kv and 25 ma. The duration of treatment was 25 seconds. Mutations in the generative nucleus are transmitted to both sperm nuclei and should be expressed in the embryo and endosperm of the F_1 kernel. Thus, the laborious task of screening large numbers of seedlings for *bz* mutants can be eliminated. The parental genotypes used in cross #2 were the same as those of cross #1. In the F_1 generation of both crosses, mutations from *Bz* to *bz*, independent of alterations at the *Sh* or *Wx* loci were saved for analysis.

Mutations from *Bz* to *bz* resulting from gross deletions including the *Bz* locus generally give *sh bz* phenotypes because of the simultaneous loss of the closely linked *Sh* locus; in addition, they may be either *Wx* or *wx*, depending on the extent of the aberration. On the other hand, putative point mutations are *Sh bz Wx* in phenotype.

If the chromosome before replication contains only one DNA double helix and if sperm nuclei

are irradiated, breaks involving single polynucleotide chains may give rise to fractional mutants following separation of the mutated and unaltered chains. However, single-chain mutations may also produce whole mutants if the uncomplementary segment is "repaired" by insertion of complementary bases into the unaffected chain. To accommodate either situation, both whole and fractional mutants must be regarded as possible intragenic alterations.

The attempt to induce mutations before the second microspore division was only partially successful; some kernels with bronze endosperm observed in cross #2 gave rise to *bz* plants and some did not. Therefore, all F_1 seeds were planted and screened for bronze at the seedling stage as in cross #1.

RESULTS

The endosperm and seedling mutants produced by X-ray treatment are listed in Table 1. Three classes of *bz* mutants were identified–*sh bz* and *Sh bz wx* types, showing loss of two dominant markers, and *Sh bx Wx* mutants, exhibiting loss of *Bz* only. Of the *Sh bz* and *sh bz* mutants detected in the endosperm, only those of *Sh bz* phenotype were classified for *Wx* versus *wx*. Bronze mutants identified at the seedling stage were scored for *Sh* and *Wx* by progeny tests. Embryo mutants of *sh bz* phenotype may include plants of *Sh bz*/sh bz* constitution in which the *Sh bz** gametophytes were nonfunctional as well as the expected—*/sh bz* class in which both dominant markers have been lost.

Of the 837 bronze mutants obtained in the embryo, the two exhibiting the *Sh bz Wx* phenotype were saved for further analysis. These are designated *bz-x1* and *bz-x2*. In addition, two *sh bz* mutants which had normal or low pollen abortion were saved. The absence of abnormal pollen indicates that a chromosomal aberration may be a minor one or may not be present; it was felt that, although the two adjacent loci were affected, intragenic events or events other than chromatin loss could be the cause of the mutations. The double mutants are designated *sh–bz-x1* and *sh–bz-x2*. All of the remaining embryo mutants exhibited high pollen abortion (indicative of a gross chromosomal aberration) and proved to be double mutants of *sh*bz*/sh bz* or *bz*wx*/bz wx* constitution. It was assumed

TABLE 1

Mutants obtained in crosses of sh bz wx × Sh Bz Wx *following irradiation of the dominant male parent*

Cross No.	Population	*sh bz*			*Sh bz Wx*			*Sh bz wx*			*bz* embryo not surviving to maturity
		Endosperm	Embryo and endosperm	Embryo	Endosperm	Embryo and endosperm	Embryo	Endosperm	Embryo and endosperm	Embryo	
1	66337	601	7	178	2	0	1	0	0	0	160
2	66620	790	42	235	7	0	1	10	0	1	212
Total	132957	1391*	49*	413*	9	0	2	10	0	1	372

* The mutants in these classes may range from deletions of the entire chromosome 9 to deficiencies which include only the *Bz* locus. If functional gametes with the mutant chromosome are not produced, only the *sh bz* phenotype will be expressed in the testcross progeny.

* The asterisk refers to any alteration which results in mutation from a dominant to a recessive phenotype.

that these plants were heterozygous for an aberration affecting the short arm of chromosome *9* and that no gene mutation had been induced. These mutants were not analyzed further. No plants mosaic for *Bz* and *bz* sectors were observed in the population of embryo mutants.

Of the four F_1 plants containing the *bz-x* or *sh–bz-x* mutations, only the *bz-x1*/ normal heterozygote appeared subnormal in stature compared to sib-plants without the mutation. The remaining three exhibited no noticeable difference in height or vigor. Seed set on ears of *bz-x1*, *bz-x2* and *sh–bz-x1* heterozygotes was slightly reduced but no conclusions can be drawn as to the extent of ovule abortion because of the possibility of imperfect pollinations. The ear produced by the *sh–bz-x2* heterozygote appeared to have normal seed set.

Pollen Analysis: Genes affecting the development and functioning of the male and female gametophytes are distributed widely on the chromosomes of maize. Generally, pollen grains associated with deficiencies of varying extent and positions are subnormal in size and devoid of starch and do not function. Cases, however, have been reported (McCLINTOCK 1944) in which deficiencies of terminal portions of the short arm of chromosome *9* are transmitted normally in male and female gametophytes. These segments are apparently devoid of genes affecting gametophyte viability.

If the *bz-x* or *sh–bz-x* mutations constitute deficiencies, segregation of aborted and normal pollen would occur in mutant/normal heterozygotes if genes affecting pollen development were also deleted. Therefore, pollen analyses were made of plants heterozygous for the four mutants. Samples of pollen from each of the mutant- and normal-sib plants were placed on slides with a drop of KI and observed under a dissecting microscope. The results are listed in Table 2. Pollen samples from two normal sibs of *Sh Bz Wx* phenotype contained an average of 6.8% grains with little or no starch. Apparently, a low frequency of abortion due to some unknown cause is characteristic of these stocks. The normal appearing

TABLE 2

Frequency of abortion and wx : Wx *ratios in pollen of plants heterozygous for the four mutants and control plants*

Plant constitution	Normal *Wx*	Normal *wx*	Sub-normal *Wx*	Sub-normal *wx*	Aborted	Percent sub-normal	Percent aborted	*wx* : *Wx* ratio in normal pollen
$\frac{bz\text{-}x1\ Wx}{bz\ \ wx}$	1500	5535	4083	417	1242	35.2	9.7	1 : 0.27
$\frac{bz\text{-}x2\ Wx}{bz\ \ wx}$	918	2439	2365	836	1594	39.3	19.6	1 : 0.38
$\frac{sh\text{-}bz\text{-}x1\ Wx}{sh\ \ bz\ \ wx}$	3551	4190	108	93	1452	2.1	15.5	1 : 0.85
$\frac{sh\text{-}bz\text{-}x2\ Wx}{sh\ \ bz\ \ wx}$	6368	6437	0	0	1546	0	10.8	1 : 0.98
$\frac{sh\ bz\ Wx}{sh\ bz\ wx}$	6233	6416	0	0	925	0	6.8	1 : 0.97

pollen varied somewhat in size in the controls but a class of distinctly smaller grains was not distinguished. However, heterozygotes involving the *bz-x1*, *bz-x2* and *sh–bz-x1* mutants produced pollen grains of normal size and those which were clearly smaller, as well as some aborted grains. Pollen grains of two types are expected since half the microspores receive a normal chromosome *9* and half receive the mutant chromosome following meiosis. It was assumed that grains with the mutant chromosome may be either subnormal or completely devoid of starch. Plants heterozygous for *sh–bz-x2* had normal pollen and a low percentage of aborted grains, but no subnormal pollen.

In heterozygotes involving *bz-x1*, *bz-x2* or *sh–bz-x1*, the high percentage of either subnormal or aborted pollen, or both, indicates that a genetic modification influencing the development of pollen arose in chromosome *9* at the time when the *bz* or *sh bz* mutations were induced.

Abortion frequencies in *sh–bz-z2* heterozygotes averaged 10.8%. This value is slightly greater than the frequency obtained in the controls; however, since the *wx* : *Wx* ratio in the normal grains from *sh–bz-x2 Wx/sh bz wx* plants is close to unity (1 : 0.98), the higher abortion frequency cannot be ascribed to the presence of the mutant chromosome.

If the assumption is made that the four mutants constitute deletions, a number of conclusions can be drawn. The low pollen abortion in *sh–bz-x2* heterozygotes indicates that genes located between *sh* and *bz* have negligible effects on pollen development. If any major gametophyte factors were located in that region, they would be eliminated along with *sh* and *bz*. Hence, genes with a conspicuous effect on pollen viability, observed in the *bz-x* mutants, appear to lie proximal to *bz*. Since *sh–bz-x1* may extend to either side of the *sh–bz* region, the gametophyte factors identified in this mutant may lie either distal to *sh* or proximal to *bz*.

Transmission studies: When a plant heterozygous for a factor adversely affecting gametophyte viability is used as a female parent in a testcross, the frequency of recovery of the deleterious allele depends on the extent to which it interferes with the development of the haploid embryo sac. Transmission by the male gametophyte is influenced by an additional factor, competition with normal grains which fall on the same style. Thus, a retarded embryo sac may eventually be fertilized, but a slow-growing pollen tube does not achieve fertilization in competition with tubes growing at a normal rate. Since the male gametophyte plays a more active role in fertilization than does the female, it is more sensitive to changes in the genome and a greater reduction in transmission frequency of a deleterious factor is expected in male than in female gametes.

In the following account, the transmission frequency of a mutation in heterozygous plants is determined by dividing the number of mutant kernels by the number of normal kernels in a given testcross progeny. In order to obtain transmission frequencies, reciprocal crosses were made of *bz-x/Bz* or *sh–bz-x/Sh Bz* heterozygotes with the appropriate tester plants. The results of these crosses are listed in Table 3.

In testcross progenies of *bz-x/Bz* plants used as male parents, no bronze kernels were observed indicating that pollen grains containing *bz-x1* or *bz-x2* either do

TABLE 3

Transmission of bz-x *and* sh–bz-x *mutants in* bz-x/Bz *and* sh–bz-x/Sh Bz *heterozygotes*
Data are obtained from testcross progenies

Heterozygotes parent	Number of plants tested	Progeny kernels *bz*	Progeny kernels *Bz*	Total	Percent transmission
$\frac{bz\text{-}x1}{Bz}$ ♂♂	5	0	7048	7048	0
$\frac{bz\text{-}x1}{Bz}$ ♀♀	7	339	1050	1389	32.3
$\frac{bz\text{-}x2}{Bz}$ ♂♂	5	0	6737	6737	0
$\frac{bz\text{-}x2}{Bz}$ ♀♀	11	1216	2115	3331	57.5
$\frac{sh\text{–}bz\text{-}x1}{Sh\ Bz}$ ♂♂	5	74	6986	7060	1.06
$\frac{sh\text{–}bz\text{-}x1}{Sh\ Bz}$ ♀♀	8	1000	1580	2580	63.3
$\frac{sh\text{–}bz\text{-}x2}{Sh\ Bz}$ ♂♂	4	3044	4022	7066	75.7
$\frac{sh\text{–}bz\text{-}x2}{Sh\ Bz}$ ♀♀	7	1459	1525	2984	95.7

not function or cannot compete with grains of normal constitution. In a testcross involving *Sh bz-x1/sh bz* heterozygotes, one *Sh bz* kernel in a progeny of 4480 proved to contain the *bz-x2* mutation. Apparently, some pollen grains which possess this mutation do function but generally, are unable to achieve fertilization in competition with normal grains.

The average transmission of *bz-x1* through the female gametophyte was 32.3%. This frequency was obtained from seven ears in which the individual transmission percentages ranged from 24.5 to 42.6. Eleven testcross ears yielded female transmission percentages of *bz-x2* ranging from 41.1 to 85.2. The average frequency was 57.5%.

In testcrosses using *sh–bz-x1/Sh Bz* heterozygotes as pollen parents, 74 kernels with the mutant phenotype occurred in a population of 7060. The resulting transmission frequency is 1.06%. Individual percentages from five plants used in this test ranged from 0.56 to 1.76. The reciprocal cross involving nine *sh–bz-x1/Sh Bz* individuals produced 2580 kernels of which 1000 were mutant in phenotype. The resulting transmission percentage of *sh–bz-x1* through the embryo sac was 63.3 while values from individual plants ranged from 55.1 to 81.7.

The haplo-viability of pollen and ovules containing *sh–bz-x2* was the greatest among the four mutants. Of six *sh–bz-x2/Sh Bz* heterozygotes tested for trans-

mission of the mutant through the pollen, two exhibited mutant transmission frequencies of 109.5% and 116.0%. Data suggested the possibility that in these individuals, a deleterious gametophyte factor was present on the normal homologue, accounting for deficiency of normal kernels in the progeny. In the four remaining plants, transmission frequencies ranged from 60.0% to 82.5% and averaged 75.7%.

In progenies from seven *sh–bz-x2/Sh Bz* heterozygotes tested for transmission of the mutant through the female gametophyte, no significant deviations from a 1:1 ratio of *sh bz* and *Sh Bz* kernels were observed. Although the average transmission was 95.7%, departures from a 1:1 ratio occurred in both directions (three ears giving an excess of *sh bz* kernels and four, a deficiency) and none was significant at the 5% level. Thus, it may be concluded that *sh–bz-x2* has no effect on megagametophyte viability.

The considerable variation of transmission frequencies through both male and female gametophytes in the four mutants can be attributed to differences in either genetic background or environment.

Effects of the mutants on crossing over: Evidence was presented by McCLINTOCK (1933) for the frequent occurrence of nonhomologous pairing in bivalents when one of the homologues carries a deletion. Nonhomologous pairing, as well as physical loss of chromatin, reduces the opportunity for crossing over between markers flanking the affected region; the extent of nonhomologous pairing and crossover reduction is related to the size of the deletion. It has been demonstrated by STADLER and ROMAN (1948) that very small deletions, *a-x1* in particular, do not noticeably disturb crossover frequencies in the affected region. Thus, normal recombination might be expected between markers flanking a minute deletion whereas a reduction in recombination should occur in heterozygotes containing a larger deficiency.

Table 4 lists the results of recombination experiments with the four mutants

TABLE 4

Recombination in pollen mother cells of
Sh bz-x Wx/sh Bz wx, sh–bz-x Wx/Sh Bz wx *and* Sh Bz wx/sh bz Wx *heterozygotes*

Plant constitution	Average percent recombination *Sh–Bz*	Average percent recombination *Bz–Wx*	Total heterozygotes involved	Total progeny
$\frac{Sh\ Bz\ wx}{sh\ bz\ Wx}$	2.29	18.6	5	8054
$\frac{Sh\ bz\text{-}x1\ Wx}{sh\ Bz\ wx}$	0.93	16.6	5	7038
$\frac{Sh\ bz\text{-}x2\ Wx}{sh\ Bz\ wx}$	0.86	18.6	5	6737
$\frac{sh\text{–}bz\text{-}x1\ Wx}{Sh\ Bz\ wx}$	0	14.2	5	7060
$\frac{sh\text{–}bz\text{-}x2\ Wx}{Sh\ Bz\ wx}$	0	19.9	6	9665

and with control plants. Heterozygotes were used as male parents since large populations can be easily produced in this manner. All experiments were performed in the same season to minimize environmental variation. Recombination values obtained from the control series indicate the normal rate of crossing over. Control values for the *Sh–Bz* region (designated region 1) ranged from 2.06% to 2.58% in individual plants. The recombination frequency based on the total population was 2.29%. In the *Bz–Wx* interval (region 2), the frequencies varied from 15.6% to 21.1% with an overall value of 18.6%.

In five plants of the genotype *Sh bz-x1 Wx/sh Bz wx*, recombination in both regions was lower than in the control plants. Frequencies of *Sh–Bz* recombination varied from 0.70% to 1.12% with 0.93% recombination in the combined progenies. Values from 15.0% to 19.0% were observed for region 2 while the frequency in the total progeny was 16.6%. The difference between the 18.6% *Bz–Wx* recombination in the control series and the 16.6% in the *bz-x1* heterozygotes is statistically significant but there is considerable overlap in values from individual ears in the two series of crosses. On the other hand, no such overlap exists in the recombination frequencies for region 1. All control plants exhibited recombination values of 2.06% or greater for this region and all *bz-x1* heterozygotes showed frequencies of 1.12% or less. Since the map distance between *Sh* and *Bz* in the control plants is 2.29 units, a decrease of 1.36 is substantial and suggestive of a deficiency. Because recombination values for the *Bz–Wx* interval are only slightly less than the control, the putative deletion includes no more than a minute segment proximal to the *Bz* locus. The nearly normal *Bz–Wx* recombination value indicates that little nonhomologous pairing occurs.

Results obtained from plants of *Sh bz-x2 Wx/sh Bz wx* constitution approximated those found in *bz-x1* heterozygotes. Five plants yielded *Bz–Wx* recombination values ranging from 13.4% to 23.0%. The frequency from the total kernel population was 18.6%, the same value found in the control data. The percentages obtained for region 1 varied from 0.60 to 0.97 while the frequency calculated from the total progeny was 0.86%. This is a significant deviation from the control. Crossing over was considerably reduced in region 1 but was not affected in region 2.

In plants carrying either of the *sh–bz-x* mutations, recombination between *Sh* and *Bz* was not observed. Crossing over between *Bz* and *Wx* in *sh–bz-x1* heterozygotes was significantly reduced. Values ranging from 11.9% to 15.7% were obtained from five plants tested while the overall percentage was 14.2. Since recombination is significantly less than in the control series, it may be concluded that either the *sh–bz-x1* deletion is large enough to induce nonhomologous pairing or that a considerable segment of chromatin proximal to *Bz* is deficient.

The *Bz–Wx* recombination frequencies based on the combined progenies of five plants in the control series and six *sh–bz-x2* heterozygotes were 18.6% and 19.9%, respectively. The difference is small but statistically significant. The recombination data from the *sh–bz-x2* heterozygotes are at variance with those from the other three mutants in that there is a significant increase in the *Bz–Wx* region. The lack of crossing over in the *Sh–Bz* region is consistent with the

deficiency hypothesis but the increase in the *Bz–Wx* region is a contradictory finding.

Cytological analyses: Heterozygotes of each of the four mutant chromosomes with a normal chromosome *9* were examined at the pachytene stage in the microsporocytes. Gross aberrations such as translocations, inversions, deletions or insertions large enough to interfere with normal pairing of homologues should be readily detected by observation of the chromosome *9* bivalent. Comparisons were made with pachytene preparations of plants containing two normal chromosomes *9*. In no case was unusual pairing observed in mutant heterozygotes.

The failure to find cytological evidence of deletions or any chromosomal aberration indicates that the origin of the four mutants did not involve gross changes in the architecture of the chromosomes possessing the mutant changes at the *bz* locus. However, the possibility that they represent very small deletions cannot be excluded on cytological grounds.

Viability of homozygotes and compounds: If a deleted segment of chromatin contains genes necessary for the survival of the zygote, individuals receiving a deficient chromosome from both the egg and sperm nuclei will not develop. To test the viability of plants homozygous for a mutant allele or heterozygous for two of the mutations, *bz-x/Bz* and *sh–bz-x/Sh Bz* compounds were selfed and intercrossed. Progeny containing only mutant chromosomes can be recognized by the *bz* phenotype.

Formation of *bz-x1* homozygotes is precluded owing to the inviability of male gametophytes carrying *bz-x1*. Selfpollinations of *bz-x2/Bz* heterozygotes were not made; inconclusive results were anticipated because of the extremely low transmission of *bz-x2* in male gametes.

Self crosses of *sh–bz-x1/Sh Bz* individuals produced less than 1000 progeny, none of which were *sh bz*, but only four would be expected (based on transmission data of the mutant through male and female gametophytes). Although the results suggest that the *sh–bz-x1* homozygote is inviable, more extensive data are needed before this conclusion can be accepted.

Four *sh–bz-x2/Sh Bz* individuals when self-pollinated, yielded progenies with 3 : 1 ratios of normal : mutant kernels; no significant deviations from this ratio were observed. Plants of *sh–bz-x2/sh–bz-x2* constitution, when self-pollinated or intercrossed, produce well-filled ears. The sporophytes are quite vigorous although no comparisons have been made with normal plants of similar background. It is apparent that the *sh–bz-x2* homozygotes are completely viable and that the mutant has no detrimental effect on sporophyte development. Data from crosses of *sh–bz-x2/Sh Bz* heterozygotes with *bz-x/Bz* or *sh–bz-x1/Sh Bz* individuals indicate that compounds of *sh–bz-x2* and the three remaining mutants are viable and the survival rate is near normal.

DISCUSSION

The results obtained in this investigation afford no evidence that X rays induce intragenic changes in maize when treatment is applied post meiotically. Three

mutants, *bz-x1*, *bz-x2* and *sh–bz-x1* were identified as minute deletions by the following criteria: (1) reduced gametophyte viability and (2) decrease in recombination between a neighboring marker and the mutant in deficiency/normal heterozygotes. Although the *sh–bz-x2* mutation exhibits only a slight reduction of transmission through the male gametophyte, this double mutant may be a deletion because of the absence of recombination between the two markers involved in the mutation. Deficiencies which include more than one locus and survive to various stages of development when homozygous have been reported in both maize and Drosophila (McCLINTOCK 1944; MULLER 1935).

Although irradiation may have produced a deficiency of both *Sh* and *Bz* which includes no viability factors, other explanations for the unusual behavior of *sh–bz-x2* must be considered. An alternative hypothesis could be proposed by which no chromatin is lost, but a suppression of the activity of the dominant alleles has occurred. A precedent for simultaneous suppression of *Sh* and *Bz* is found in the work of McCLINTOCK (1953, 1956). In her extensive studies of the *Ds-Ac* mutator system, she found cases in which a *Ds* element, transposed just to the left (distal) of *Sh*, induced a simultaneous loss of *Sh* and *Bz* expression.

The assumption that a controlling element, either X ray-induced or already present in the stock, was inserted next to the *Sh* and *Bz* loci faces the difficulty of accounting for the lack of recombination between *Sh* and *Bz* but does explain the viability of the homozygotes. If the assumption is made that the *Sh–Bz* region is deficient, the suppression of recombination between the two loci is understandable. The viability of *sh–bz-x2* homozygotes must mean that no vital loci are situated in the missing segment and the normal functioning of gametophytes carrying the mutant also indicates, on a deficiency hypothesis, that no essential gametophyte genes reside in the deficient segment. However, the failure of *sh–bz-x2* to reduce recombination in the *Bz–Wx* region argues against the deficiency hypothesis and appears to favor that of gene inhibition. No firm conclusions as to the nature of the *sh–bz-x2* mutant are possible.

The results obtained in the present study and in the earlier work by STADLER and ROMAN indicate that X rays do *not* cause intragenic mutations in maize. Although some recessive mutations induced at unmarked loci by X rays give normal F_2 ratios as expected from intragenic mutations (STADLER, 1941), such findings are not contradictory to the conclusion stated above. These mutants may be deletions with no effect on gametophytic transmission or zygotic viability.

In the present study, the *bz*/bz* plants which survived to maturity were backcrossed to the recessive parent regardless of whether defective pollen was present or not. By this procedure, every transmissible change involving only the *Bz* locus was recovered. If the sterility in a plant resulted from an independent aberration and the bronze expression was due to an intragenic mutation at the bronze locus, the backcross ear would segregate for the *Sh* and *sh* phenotypes. Since highly sterile plants surviving to maturity produced ears bearing kernels of *sh bz* or *bz wx* phenotype only, the cause of sterility and the apparent double mutation in each case may be ascribed to an aberration in chromosome *9*. The alternative possibility of simultaneous point mutations at two gene loci in chromosome *9*

accompanied by an aberration in a second chromosome is considered highly improbable and these plants were not subjected to further analysis.

Although none of the *bz* mutations proved to be intragenic alterations, such changes could have escaped detection in plants which did not survive to maturity. Of the 837 mutants obtained in the embryo, 372 failed to produce ears and/or tassels. Hence, almost half the plants could not be tested for the modification causing expression of the bronze phenotype.

Studies in Neurospora by WEBBER and DE SERRES (1965) indicate that with X-irradiation, deletions increase as the square of the dose whereas point mutations increase linearly with dose. In light of these induction kinetics, the possibility must be considered that in maize, a dose of 1000r is above the optimal level for inducing point mutations and favors the recovery of deletions and other two-hit phenomena.

The work of RUSSELL and RUSSELL (1959, 1960) with mice indicates that some mutations produced when spermatogonial cells are irradiated may be intragenic, although the nature of these mutations has been questioned by WOLFF (1967). RUSSELL and RUSSELL state that many of the gross aberrations induced in the spermatogonial stage are eliminated during the meiotic cycle. Consequently, when cells are irradiated at premeiotic stages, the selective factors operating during meiosis should favor the recovery of intragenic rather than extragenic changes. On the assumption of a similar filtering mechanism in maize, the irradiation of sporogenous tissue should increase the proportion of putative gene mutations.

It is possible that intragenic mutations induced by X rays in the present study were not detected because of the operation of a DNA-repair mechanism. PETTIJOHN and HANAWALT (1964) reported that in *E. coli* (B/r), an error-correcting mechanism exists which excises and replaces damaged single-strand regions, using the undamaged DNA strand as a template. Although this system accounts for the repair of alterations induced by UV and nitrogen mustard (HANAWALT and HAYNES 1965), additional evidence (HAYNES, PATRICK and BAPTIST 1964) indicates that X ray-induced changes can also be corrected.

It may be inferred that an error-correcting process akin to the one in *E. coli* exists in higher organisms. In fact, the high mutability of certain loci may reside in the inability of a repair enzyme to recognize alterations of the base sequences in some cistrons.

Although the present investigations on the *Bz* locus and comparable studies by STADLER and ROMAN on the *A* locus have revealed no instance of the induction by X rays of an intragenic mutation, it cannot be concluded that ionizing radiations are incapable of producing this type of gene transformation. The evidence for true gene mutation as a consequence of X-irradiation in lower forms is so compelling that it seems unlikely that similar mutations would not occur in maize if some technique could be found to efficiently screen much larger populations than is now feasible. Nevertheless, in fairly extensive experiments at both the *A* and *Bz* loci, the only mutations induced were those involving deficiencies or possibly a gene-control system. It cannot be denied that X rays are not an

extremely effective mutagenic agent but it is clear that the vast majority of induced changes are of a relatively gross nature and any intragenic mutations may be swamped by the much greater number of extragenic modifications.

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SUMMARY

A study was made of the effects of X-irradiation on the *Bz* locus in chromosome *9* of maize. Pollen of *Sh Bz Wx* homozygotes was X-irradiated and applied to silks of *sh bz wx* tester plants. Of 132957 F_1 plants, 837 exhibited the bronze phenotype. Two plants of *Sh bz*Wx/sh bz wx* constitution (the *bz-x1* and *bz-x2* mutants) were selected for more intensive study as well as two plants with nearly normal pollen in which both *Sh* and *Bz* markers were mutated (*sh–bz-x1* and *sh–bz-x2*). All of the remaining plants proved to be double mutants of *sh* bz*/sh bz* or *bz* wx*/bz wx* constitution with high pollen abortion. They were assumed to be heterozygous for deletions in chromosome *9* and were not analyzed further. The *bz-x1*, *bz-x2* and *sh–bz-x1* mutants were classified as small deletions on the basis of results from pollen analysis, gametophytic transmission and crossover studies. The nature of *sh–bz-x2* cannot be categorically defined because of contradictory evidence from genetic tests. A deficiency hypothesis and an interpretation of *sh–bz-x2* as a suppressed state of *Sh* and *Bz* are considered.

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