6. The effects of x-rays on the bronze locus.

Stadler and Roman (1948) found no intragenic changes at the A_1 locus as a result of x-irradiation. Although their tests on induced mutation were extensive, they were confined to a single locus. The conclusion that x-rays do not induce intragenic changes in maize requires further support from studies on other loci. The bronze locus on the short arm of chromosome 9 has been chosen as a favorable gene for mutation experiments. Chromosome 9 carries the markers sh, 2 map units to the left of bz, and wx, 21 map units to the right.

Two crosses involving Bz stocks were made. In cross #1, pollen from plants homozygous for A_1 A_2 C R^r Pr B Pl Sh Bz Wx was x-rayed with a dose of 1000r and used to pollinate plants homozygous for A_1 A_2 C R^g Pr b pl sh bz wx. R^r was the contamination marker. In cross #2, the male parent was irradiated with the same dosage approximately 13-19 days prior to anthesis. This was suggested by Caspar as an attempt to induce mutations in the generative nucleus before the second microspore division. The resulting F_1 seeds would carry the same mutation in the embryo and endosperm, thus eliminating the laborious task of screening large numbers of seedlings. The male parent in this cross was homozygous for A_1 A_2 C R^r Pr B Pl Sh Bz Wx Og and was crossed to plants homozygous for A_1 A_2 C R^r Pr B Pl sh bz wx og. Og was the contamination marker in this case.

Mutants from Bz→bz due to gross chromosomal aberrations will appear as sh bz in phenotype and may be either Wx or wx, depending on the extent of the aberration. Any putative point mutations will be Sh bz Wx in phenotype.

Bronze seedlings carrying A_1 A_2 B Pl R^r show no red pigmentation in the stem, coleoptile, roots or leaf tips. In cross #1, F_1 seedlings of this phenotype were selected and transplanted. All bronze mutants were classified for pollen abortion and backcrossed to a sh bz wx stock. The presence of pollen abortion indicates that a chromosomal aberration exists; however, it is not necessarily in chromosome 9. Therefore, kernels from this backcross were screened for Sh bz Wx phenotypes as an indication of a possible point mutation at the bronze locus.

In cross #2, the attempt to cause mutations before the second microspore division was only partially successful. Some bronze mutations in the endosperm corresponded with bronze mutations in the embryo, and some did not. Therefore, all seeds are being planted and screened as in cross #1. The following table contains results from

these two crosses.

Table 1

Cross #	Total F_1 pop.	sh bz mutants		Sh bz Wx mutants		Fractionals and/or mosaics in endosperm
		endo.	emb.	endo.	emb.	
1	66,337	608	345**	2	1	272
2*	26,151	375	incomplete counts	4	incomplete counts	80

* Tabulations on this cross have not been completed. Partial results are indicated.

** These mutants could not be classified for sh since none were transmitted.

The one bronze mutant from cross #1 (Table 1) carrying the Sh and Wx markers is designated bz-X_1. The F_1 plant was normal in appearance and exhibited no aborted or sub-normal pollen. The plant was backcrossed to a sh bz wx tester. When used as the female parent, it yielded a small ear with 15 Sh bz Wx seeds, 20 sh bz Wx seeds and only 1 sh bz wx seed. Seed set was poor. Further tests will be made to determine the cause of this unexpected ratio.

The following data were obtained using the above F_1 plant as the male parent in crosses to a sh Bz wx stock.

Table 2

Total pop.	Recomb. classes		Non-recomb. classes		Recombination
	Sh wx	sh Wx	sh wx	Sh Wx	Sh-Wx
373	2	38	333	0	10.7%

The recombinants must be backcrossed to determine if they are carrying the mutant region. If such is the case, transmission of the markers linked with the bz-X_1 allele will be sub-normal.

Average recombination between sh and wx is 21% (Emerson, Beadle and Fraser, 1935); however, control values have not been determined for the stocks involved in this experiment. In the second backcross using 5 of the 15 Sh bz Wx kernels, the following results were obtained.

Table 3

	Sh bz-X_1 Wx / sh bz wx ♀ x sh bz wx ♂				
	Recomb. classes		Non-recomb. classes		% recomb.
Family	Sh wx	sh Wx	Sh Wx	sh wx	Sh-Wx
851-1	1	26	14	111	17.8
851-2	7	28	5	71	31.5
851-3	5	26	3	112	21.2
851-4	2	33	2	169	17.0
851-5	6	19	5	128	15.8
Total	21	132	29	591	19.8
	sh bz wx ♀ x Sh bz-X_1 Wx / sh bz wx ♂				
851-1	9	80	0	449	16.5
851-2	5	104	0	722	13.1
851-3	-	--	-	--	---
851-4	1	25	0	84	23.6
851-5	7	61	0	554	10.9
Total	22	270	0	1809	13.9

An additional group of bz-X_1 plants which were used only as male parents in crosses to sh bz wx testers yielded an average recombination value of 12.8%. One bz-X_1

plant which was not included in this group exhibited greater than 23% recombination between Sh and Wx. Only two Sh bz Wx seeds were recovered, probably resulting from double crossovers. Therefore the aberrant chromosome was present. The total number of seeds obtained using this plant as a male parent was 1121. A value greater than 23% was obtained also from the cross of sh bz wx ♀ x 851-4 ♂ (Table 3); however this was based on a progeny of only 110, indicating that the resulting percentage of recombination may not be significant.

In the female gametes, a wide range of recombination between Sh and Wx was observed (Table 3). The average value was 19.8% as compared to 13.9% in the male gametes from the same plants. A reduction in crossing over due to non-homologous pairing is expected if a deletion is present; however, if one can extrapolate from data of other aberrations, (Dempsey, MNL 35: 63, and Burnham, MNL 35: 86) the greater recombination value would be expected in the male gametes.

Further genetic and cytological observations will be made to determine the nature of this mutation.

The work done so far on a population of 92,488 corroborates the evidence of Stadler and Roman that intragenic changes do not occur in maize as a result of x-rays, when the plants are irradiated post-meiotically.

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