EFFECTS OF PRETREATMENT WITH X RAYS ON PARAMUTABILITY OR PARAMUTAGENICITY OF CERTAIN *R* ALLELES IN MAIZE¹

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LINDEN (1963) first reported that the paramutation process in maize was radiosensitive. He showed that when pollen from plants homozygous for either paramutable standard *R^r* or paramutagenic *R* alleles such as *Rst* (stippled aleurone) and *R^{mb}* (marbled aleurone) was gamma-irradiated, paramutation was altered in the heterozygotes into which these alleles were subsequently introduced. Evidence is presented here confirming and extending LINDEN's observations.

MATERIALS AND METHODS

The materials, methods, and terminology used in this experiment have been described in part in the preceding article (SHIH and BRINK 1968). In addition to the materials mentioned in that report, a group of self-colored aleurone, green anther mutants (*R^{sc}*) from the stippled allele (*Rst*) were employed. Among these *R^{sc}* alleles, *R^{sc}₆₁*, *R^{sc}₈₅* and *R^{sc}₁₁₃* alleles were non-paramutagenic, *R^{sc}₁₁₀*, *R^{sc}₁₁₄* and *R^{sc}₁₁₉* were weakly paramutagenic, and *R^{sc}₇₇*, *R^{sc}₁₃₂* and *R^{sc}₁₃₈* were strongly paramutagenic (McWHIRTER and BRINK 1962). Also, two independently occurring *r^r*(I) mutants, *r^r*(I)₃ (weakly paramutagenic) and *r^r*(I)₅ (strongly paramutagenic), isolated from testcrosses of *R^rRst* heterozygotes were utilized. The *r^r*(I) mutants condition red anthers. They give colorless aleurone in one dose and near-colorless aleurone in two or three doses, and are assumed to have arisen by recombination between the *R^r* and *Rst* alleles.

EXPERIMENTAL RESULTS

*Reduction in paramutability of X-irradiated *R^r* alleles:* Seeds and pollen from *R^rR^r* homozygotes were X-irradiated with 10,000 r and 1,200 r, respectively, and were then used to obtain crosses with unirradiated *RstRst* plants. The paramutability of the irradiated *R^r* alleles was measured by testcrossing the resulting *R^rRst* heterozygotes on *r^or^o* plants.

The *R^r* alleles showed no detectable change in paramutability after seed treatment (Table 4). This result could be due to the insufficiency of the X-ray dose applied. In contrast, 27% of the plants resulting from pollen treatment showed a reduction in the paramutability of *R^r*. Distributions of the mean aleurone scores of the testcross kernels derived from the control *R^rRst* family and the three treated *R^rRst* families carrying X-irradiated *R^r* alleles are depicted in Figure 1. Each treated family represents the irradiated *R^r* pollen from one *R^rR^r* plant. The increases in *R^r^or^o* aleurone pigmentation of the three treated families are relatively uniform after irradiation, and each family shows a highly significant

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FIGURE 1.—Distributions of testcross ears from matings on $r^g r^g$ females of $R^r R^{st}$ heterozygotes carrying R^r alleles X-irradiated with 1,200r in pollen or unirradiated. The marked points represent the percent of testcross ears falling between the class limits shown for aleurone color. The three treated families are replicates and are derived from the same stock as the untreated control. All three treated families show significantly lower paramutability than the control.

reduction in paramutability (Table 1). In no case, however, was paramutability completely lost.

Heritability and R locus dependence of the induced reduction in paramutability of R: X-irradiated R^g alleles (pollen, 1,200 r) were tested for heritability of the altered paramutability and for R locus dependence of the change in the progeny of heterozygotes with unirradiated standard R^r . Pollen from the $R^r R^g$ plants was placed on $R^{st} R^{st}$ silks, and the $R^r R^{st}$ and $R^g R^{st}$ heterozygotes thus obtained were then testcrossed on $r^g r^g$ plants. In this experiment, the irradiated R^g alleles were propagated for one generation in heterozygotes with standard R before their level of action was tested.

TABLE 1

Mean aleurone color scores of R^r $r^{st} r^{st}$ kernels derived by pollinating $r^{st} r^{st}$ plants with pollen from $R^r R^{st}$ heterozygotes carrying X-irradiated and unirradiated R^r alleles

Male family No.	Treatment	Number of testcross matings	Mean score	t value
K3219	Control	77	1.59	...
K3222	Treated	124	1.85	4.64*
K3227	Treated	105	1.80	4.10*
K3231	Treated	161	1.84	4.68*

* Highly significant ($P < .01$).

TABLE 2

Mean aleurone color scores of $R^r r^g r^g$ and $R_6^g r^g r^g$ kernels from testcrosses of $R^r R^{st}$ and $R_6^g R^{st}$ plants derived from $R^r R_6^g$ heterozygotes carrying X-irradiated and unirradiated R_6^g alleles

Male family no.	$R^r r^g r^g$			$R_6^g r^g r^g$		
	Number of testcross matings	Mean score	t value	Number of testcross matings	Mean score	t value
Control						
K3439	2	2.52		2	1.79	
K3440	2	1.97		3	2.06	
K3441	3	2.02		2	1.50	
K3442	3	2.13		2	1.84	
K3443	3	2.45		2	1.84	
K3444	3	2.24		2	1.85	
K3445	2	2.02		3	1.71	
Mean		2.19			1.08	
Treated						
K3446	3	2.00	0.85†	2	2.11	5.02*
K3447	2	1.72		3	2.04	
K3448	2	2.70		4	2.23	
K3449	3	2.57		2	2.50	
K3450	1	1.66		4	2.37	
K3451	3	1.87		4	2.08	
K3452	1	2.30		3	2.05	
K3453	2	2.51		4	2.04	
K3454	1	2.06		1	2.27	
K3455	3	1.91		4	2.20	
K3456	3	1.81		3	2.38	
K3457	2	1.68		2	2.08	
K3458	3	1.98		2	2.25	
K3459	3	2.17		3	2.68	
Mean		2.07			2.23	

* Highly significant ($P < .01$).

† Not significant ($P > .05$).

The mean aleurone color scores of the two resulting classes of kernels (Table 2) indicate that the irradiated R_6^g alleles retained to a significant degree the reduced sensitivity to R^{st} action. Paramutability of the untreated R^r alleles, however, remained unchanged although they had been made heterozygous with the irradiated R_6^g alleles. This observation provides evidence that the X-ray-induced reduction in paramutability of R is R locus dependent.

Other tests (Table 3) show that there was no significant difference in aleurone pigmenting ability of R^r alleles derived from $R R^{st}$ heterozygotes in which the R alleles had passed through plants carrying r alleles from treated and untreated pollen (1,200 r). It is concluded, therefore, that the X-ray-induced reduction in paramutability of R was not mediated by genetic factors at other loci. The changes induced at the R locus, however, may be direct, or indirect via the cytoplasm.

TABLE 3

Mean aleurone color scores of $R'r^g$ kernels derived from $R R^{st}$ heterozygotes involving R alleles which had been passed through plants carrying X -irradiated and unirradiated r alleles

Number of families†	Treatment	Aleurone	Mean score	<i>t</i> value
8	Control	$R'r^g$	2.66	1.08*
19	Treated		2.52	
6	Control	$R_r^{g'} r^g$	3.02	1.19*
16	Treated		2.84	

* Not significant ($P < .05$).

† Each family is derived from one R^r allele which had been passed through a heterozygote carrying one X -irradiated or unirradiated r allele. On the average, 7 testcross matings were made in each family.

Changes in paramutagenicity in X -irradiated R^{st} alleles: Homozygous $R^{st}R^{st}$ seeds and pollen from $R^{st}R^{st}$ plants were X -irradiated with 10,000 r and 1,200 r, respectively, and were used to obtain crosses with unirradiated R^rR^r plants. The paramutagenicity of the irradiated R^{st} alleles was then measured by testcrossing the R^rR^{st} heterozygotes on $r^g r^g$ plants.

The R^{st} allele showed no detectable changes in paramutagenicity after irradiating $R^{st}R^{st}$ seeds. Again the X -ray dose applied may not have been high enough to bring about detectable changes (Table 4). Among seven families (Table 5) derived from R^rR^{st} heterozygotes carrying R^{st} alleles irradiated in pollen, on the

TABLE 4

Mean aleurone color scores of paramutant R^r alleles derived from R^rR^{st} heterozygotes carrying R^r or R^{st} alleles irradiated and unirradiated with 10,000r of X rays at the seed stage before being crossed to $R^{st}R^{st}$ or R^rR^r . Pollen of R^rR^{st} plants carrying irradiated and unirradiated R^r or R^{st} alleles was put on silks of an $r^g r^g$ line.

Number of families tested*	Treatment	Number of testcross matings	Mean score	<i>t</i> value
9	Control	32	1.78	0.75†
17	Treated (R^r)	54	1.70	
9	Control	56	1.56	0.75†
17	Treated (R^{st})	79	1.50	
3	Control	15	1.73	0.54†
9	Treated (R^{st})	56	1.67	

* Each family is derived from one treated or untreated $R^{st}R^{st}$ seed. The two respective groups of R^{st} allele treatment shown in the table comprising treated and control entries were derived from sib W22 inbred $R^{st}R^{st}$ ears. Furthermore, with each group the control and treated seeds were sibs.

† Not significant ($P > .05$).

TABLE 5

Mean aleurone color scores of $R^r r^g r^g$ kernels derived by pollinating $r^g r^g$ plants with pollen from $R^r R^{st}$ heterozygotes carrying X-irradiated and unirradiated R^{st} alleles

Family No.§	Treatment	Number of testcross matings	Mean score	t value
K3247	Control	57	1.86	
K3251	Treated	22	2.21	2.22*
K3253	Treated	37	1.75	0.58‡
K3255	Treated	31	1.68	1.27‡
K3257	Treated	73	2.91	7.65†
K3264	Treated	51	1.84	0.20‡
K3267	Treated	58	1.81	0.55‡
K3269	Treated	45	1.74	1.35‡

* Significant ($P < .05$).

† Highly significant ($P < .01$).

‡ Not significant ($P > .05$).

§ Each family is derived from the R^{st} pollen grains produced by one $R^{st}R^{st}$ plant. The 8 $R^{st}R^{st}$ plants represented are sibs.

other hand, one family (K3251) showed a significant and another one (K3257) showed a highly significant, reduction in paramutagenic action. Each family in Table 5 represents the pollen produced by one $R^{st}R^{st}$ plant, and each testcross mating was based on one X-irradiated or unirradiated R^{st} pollen grain. About 18% of the testcross ears of K3251 and 30% of the testcross ears of K3257 were higher in aleurone pigmentation than the control. The methods used to calculate these frequencies have been described in the preceding article (SHIH and BRINK 1968). Three families based on treated pollen showed a reduced aleurone pigmentation, namely, K3253, K3255, and K3269. Statistical analyses, however, do not show that the changes in the R^{st} action in these latter cases are significant. The distributions of the mean aleurone scores of the testcross matings of families K3251 and K3257 and of the control family K3247 are illustrated in Figure 2.

Persistence of the reduction in paramutagenicity of the significantly affected R^{st} alleles: The $R^{st}r^g$ kernels used in this experiment were selected from the darker testcross ears and the lighter testcross ears, respectively, in the control K3247 family and the treated K3257 family listed in Table 5. The paramutagenicity of these R^{st} alleles was again evaluated after the alleles were entered in heterozygotes with standard R^r . The mean scores of the $R^{rr}r^g r^g$ testcross kernels derived from the four groups of $R^r R^{st}$ heterozygotes are shown in Table 6.

The evidence summarized in Table 6 shows that the reduced paramutagenicity of the treated R^{st} alleles (group B) was heritable, since the progeny also showed a highly significant reduction in paramutagenicity compared to the R^{st} alleles derived from the other sources (groups A, C, and D). The R^{st} alleles in the progeny in group D showed the same strong paramutagenicity in $R^r R^{st}$ heterozygotes as did the parent alleles. That there was no difference in paramutagenicity between the alleles of group D and the control R^{st} alleles in groups A and C proves that the irradiated R^{st} alleles in group D were not affected by X irradiation.

FIGURE 2.—Distributions of testcross ears from matings on $r^g r^g$ females of $R^r R^{st}$ heterozygotes carrying R^{st} alleles X-irradiated with 1,200r in pollen or unirradiated. The marked points represent the percent of testcross ears falling between the class limits shown for aleurone color. The two treated families are replicates and are derived from the same stock as the untreated control. The two treated families represented show decreased paramutagenic action of the R^{st} allele.

TABLE 6

Mean aleurone color scores of $R^r r^g R^r r^g$ kernels derived from the testcrosses of $R^r R^{st}$ heterozygotes involving R^{st} alleles from X-irradiated and unirradiated sources

Male family no. of G_1	Tested R^{st} allele derived from the higher scoring G_1 ears			Tested R^{st} allele derived from the lower scoring G_1 ears		
	Mean score of selected G_1 ear	No. of G_2 testcross matings	Mean score of G_2 family	Mean score of selected G_1 ear	No. of G_2 testcross matings	Mean score of G_2 family
K3247	Control					
	2.46	14	1.81	1.66	14	1.64
	2.24	11	1.59	1.10	9	1.41
K3257	2.50	10	1.36	2.02	8	1.44
	Treated					
	4.48	9	4.06	1.44	10	1.50
	4.70	9	3.11	1.30	9	1.51
	5.44	7	3.46	1.30	10	1.35

A vs B, $t=6.39^{**}$; C vs B, $t=7.32^{**}$; D vs B, $t=7.41^{**}$;

C vs D, $t=0.14^*$; A vs C, $t=0.97^*$; A vs D $t=0.95^*$.

* Not significant ($P > .05$).

** Highly significant ($P < .01$).

The suggestion that the original R^{st} allele in the $R^{st}R^{st}$ plants from which all of the above-mentioned treated R^{st} alleles were derived had changed before the test was made so that this R^{st} line subsequently varied widely in paramutagenicity can thus be ruled out as an explanation of the highly reduced paramutagenicity of the R^{st} alleles in K3257.

In group B, which showed weak paramutagenicity, the progeny (mean score = 4.06) of one of the affected R^{st} alleles was significantly less paramutagenic ($t = 2.81$, $P < .05$) than was the progeny (mean score = 3.11) of a second affected allele. That the paramutagenicity of these affected alleles was different precludes the possibility that mutation had previously occurred in their common parent allele, namely, one of the alleles in the $R^{st}R^{st}$ plant from which the R^{st} pollen was obtained which was then X-irradiated. Had the change in paramutagenicity of these affected alleles resulted from such a mutation the progeny would have shown the change in paramutagenicity but would not have shown significant differences in paramutagenicity among themselves. It is concluded that the weak paramutagenicity of the R^{st} alleles in question was heritable and was X-ray-induced.

Effects of X-irradiation on paramutagenicity of R^{sc} and $r^r(I)$ alleles: It will be recalled that R^{sc} symbolizes an allele derived by mutation from stippled (R^{st}) which gives self-colored aleurone. Factors designated $r^r(I)$ are derived from R^rR^{st} heterozygotes and are assumed to result from recombination within the locus. Independently occurring mutants of these two classes are distinguished by different numerical subscripts.

The paramutagenic R^{sc} and $r^r(I)$ stocks tested were crossed to standard R^rR^r or $R^g_6R^g_6$ homozygotes, used as females, and the heterozygotes thus obtained were then testcrossed on r^gr^g plants. The resulting testcross $R^{r'}r^gr^g$ or $R^g_6'r^gr^g$ kernels were examined for changes in pigmentation level that could have resulted from alteration in paramutagenicity of the tested R^{sc} and $r^r(I)$ alleles.

In Table 7, each family (comprising 6 or 7 testcross matings) was derived from a treated (14,000 r) or untreated $R^{sc}R^{sc}$ or $r^r(I)/r^r(I)$ seed. The mean score of each family was compared to the scores of the two control families. The 68 families derived from the 68 treated $R^{sc}R^{sc}$ seeds, including nine R^{sc} alleles initially differing in level of paramutagenicity, were found to be insensitive to X irradiation. The R^{sc}_{110} alleles, weakly paramutagenic in standard form and derived from one X-irradiated $R^{sc}_{110}R^{sc}_{110}$ seed, and the R^{sc}_{132} alleles, strongly paramutagenic in standard form and derived from one X-irradiated $R^{sc}_{132}R^{sc}_{132}$ seed, were exceptional, however, and showed increases in paramutagenicity. The data for these two cases are shown separately from their respective groups in Table 7. Two paramutagenic $r^r(I)$ alleles also were studied. Paramutation of R^g_6 was reduced in the $R^g_6/r^r(I)_5$ heterozygotes when the strongly paramutagenic $r^r(I)_5$ allele was X-irradiated at the seed stage with 14,000 r. Such a reduction did not occur, however, in R^g_6 heterozygotes carrying the irradiated weakly paramutagenic $r^r(I)_3$ allele.

Further paramutagenicity tests with the R^{sc}_{110} and R^{sc}_{132} alleles: Progeny tests were made, each based on a single male family of the two entries in Table 7

TABLE 7

Mean aleurone color scores of $R^r r^s r^g$ kernels from testcrosses of $R^r R^{sc}$ and $R_6^g/r^r(I)$ heterozygotes carrying R^{sc} or $r^r(I)$ alleles X-irradiated or unirradiated at the seed stage

Number of male families	Allele	Number of testcross matings	Mean score	<i>t</i> value
Non-paramutagenic R^{sc} alleles				
2 (Control)	R_{61}^{sc}	10	6.94	
6 (Treated)		37	6.86	1.18†
2 (Control)	R_{85}^{sc}	12	6.92	
6 (Treated)		41	6.89	0.54†
2 (Control)	R_{113}^{sc}	12	6.90	
8 (Treated)		49	6.82	1.52†
Weakly paramutagenic R^{sc} alleles				
2 (Control)	R_{114}^{sc}	13	5.50	
12 (Treated)		47	5.57	0.37†
2 (Control)	R_{119}^{sc}	18	5.30	
4 (Treated)		24	5.37	0.29†
2 (Control)	R_{110}^{sc}	14	5.73	
3 (Treated)		24	5.66	0.35†
1 (Treated)		8	5.28	2.58*
Strongly paramutagenic R^{sc} alleles				
2 (Control)	R_{77}^{sc}	13	2.66	
4 (Treated)		25	2.63	0.11†
2 (Control)	R_{132}^{sc}	15	2.72	
13 (Treated)		99	2.64	0.44†
1 (Treated)		5	1.57	3.88**
2 (Control)	R_{138}^{sc}	16	4.15	
10 (Treated)		72	3.86	0.77†
Weakly paramutagenic $r^r(I)$ allele				
2 (Control)	$r^r(I)_3$	15	4.15	
8 (Treated)		57	4.14	0.13†
Strongly paramutagenic $r^r(I)$ allele				
2 (Control)	$r^r(I)_5$	16	2.63	
10 (Treated)		70	2.90	2.13‡

** Highly significant ($P < .01$).

* Significant ($P < .05$).

† Not significant ($P > .05$).

‡ Not significant at the 5% level but significant at the 10% level ($.10 > P > .05$).

which appeared to show enhanced paramutagenic action as a result of X irradiation, namely, R_{110}^{sc} and R_{132}^{sc} . Plants grown from testcross seed representing the respective control and treated families were again crossed with the standard R^r stock. The resulting R_{110}^{sc} and R_{132}^{sc} heterozygotes with R^r were then testcrossed

TABLE 8

Mean aleurone color scores of $R^r r^{sc}$ kernels derived from the testcrosses of $R^r R^{sc}$ heterozygotes involving R^{sc} alleles from X-irradiated and unirradiated sources

Treatment	Allele	Mean score of G_1 family	Mean score of selected G_1 ear for further test	Number of testcross matings of G_2	Mean score of G_2	<i>t</i> value (Control G_2 vs Treated G_2)
Control	R^{sc}_{110}	5.73	5.56	8	5.12	
Treated		5.28	4.22	23	5.25	0.48†
Control	R^{sc}_{132}	2.72	2.68	27	2.87	
Treated		1.57	1.30	27	2.64	2.09*

* Significant ($P < .05$).

† Not significant ($P > .05$).

again on $r^g r^g$ females. The mean scores for the $R'' r^g r^g$ testcross kernels obtained are shown in Table 8.

The results show that the initially observed increase in paramutagenicity of the treated R^{sc}_{110} stock (G_1) did not persist in the progeny (G_2). In case of the R^{sc}_{132} allele, on the other hand, the progeny again gave evidence of increased paramutagenicity in the treated line, although the difference between the treated and control stocks was much less in the progeny (G_2) than in the parent (G_1).

DISCUSSION

It was shown in the preceding article (SHIH and BRINK 1968) that the partial derepression often induced by X rays in highly repressed R alleles can be satisfactorily accounted for in terms of loss of elements making up a repressor segment adjacent to the R gene. The results were in accord with expectation on the basis of (1) STADLER's (1941) earlier finding that high energy radiations induce deficiencies but not gene mutations, in maize, (2) changes which consisted of losses of repeating units, termed metameres, from a postulated repressor segment at the R locus whose size regulated the level of expression of the R gene. The results obtained in the present study, with one possible exception, are explainable in the same terms.

LINDEN (1963) reported that sensitivity to paramutation of R^r can be reduced by treating R^r pollen with gamma-rays before the allele is made heterozygous with either of the paramutagenic factors R^{st} or R^{mb} . This finding was confirmed in the present investigation; 27% of the plants carrying an R^r allele that had been irradiated with X rays in pollen subsequently showed a reduction in paramutability in R^{st} heterozygotes. Paramutability was not completely lost in any case after irradiation. Pretreatment of R^r in seeds, however, did not alter sensitivity of the allele to R^{st} action. This result could be attributed to an insufficient dosage of X irradiation. The changes induced in the pollen were shown to be heritable, and also to involve the R locus.

LINDEN (1963) also observed that when the R^{st} allele was gamma-irradiated

in pollen the induced changes in paramutagenicity were regularly to a lower level of action. This regularity did not hold, however, in experiments with the similarly paramutagenic marbled (R^{mb}) factor. Paramutagenicity was lost in some treated R^{mb} stocks but was raised to a higher level than in the control in other treated lines. Several instances were observed in the present investigation in which paramutagenicity of the stippled (R^{st}) allele was significantly reduced, but not entirely lost, by X irradiation. None of the observed instances of small changes in R^{st} in the opposite direction, namely, increased paramutagenicity, were demonstrably real. R^{st} in standard form, however, is highly paramutagenic, so that an increase in level of action is not easily detected. The paramutagenicity of the $r^r(I)_5$ but not the $r^r(I)_3$ allele was reduced also by treatment of $r^r(I)/r^r(I)$ seeds with 14,000 r. Among nine R^{sc} alleles tested two instances were found, however, in which level of paramutagenic action seemingly was raised by X irradiation of $R^{sc}R^{sc}$ seeds. One of these alleles, R^{sc}_{110} , is weakly paramutagenic in standard form. Testcrosses on $r^g r^g$ females of R^r/R^{sc}_{110} control plants, based on crosses of a standard R^r stock with individuals reared from untreated homozygous R^{sc}_{110} seeds, gave an average aleurone color score of 5.73. Three groups of R^r/R^{sc} plants based upon homozygous R^{sc}_{110} seeds pretreated with 14,000 r gave an average score of 5.66, which does not differ significantly from the control value. One group of R^r/R^{sc}_{110} plants based on another homozygous R^{sc}_{110} seed pretreated with 14,000 r, however, gave a testcross score, following matings on $r^g r^g$ females, of 5.28. This value is significantly lower at the 5% level, than the control score. As mentioned earlier, a progeny test using testcross kernels from the latter group, however, showed that the initially observed difference between treated and controlled stocks did not persist. It may be concluded, therefore, that X irradiation did not alter the level of action of the weakly paramutagenic R^{sc}_{110} allele.

A possibly valid, but still uncertain, instance of increase in level of paramutagenic action following X-irradiation is afforded by the results with R^{sc}_{132} , a strongly paramutagenic mutant from stippled. The control score following $r^g r^g \text{♀} \times R^r/R^{sc}_{132} \text{♂}$ testcrosses in this case was 2.72. Thirteen among fourteen R^r/R^{sc}_{132} groups of plants each based on an $R^{sc}_{132}/R^{sc}_{132}$ seed pretreated with 14,000 r, similarly tested, gave an average score of 2.64, a value which is not significantly different from that of the control. The other R^r/R^{sc}_{132} plants used in this test, based on the remaining single irradiated $R^{sc}_{132}/R^{sc}_{132}$ seed, in contrast, gave an average testcross score of 1.57. The seeming increase in paramutagenicity over the control in this case is highly significant statistically. A progeny test based on the testcross kernels in the latter case showed significantly higher paramutagenicity of the treated stock than the control. The difference in score, however, was only 0.23 in the progeny as compared with 1.15 in the parental generation. The meaning of the marked reduction in the differences from parent to progeny is obscure, and raises a doubt whether in this instance X irradiation has effected a real increase in paramutagenicity.

Sensitivity of standard R^r to R^{st} action and the level of pigment producing action of repressed R alleles, if affected at all, have been found in the present experiments to be changed by high energy irradiation in one direction only. The

evidence on induced change in paramutagenicity of paramutagenic alleles, such as R^{st} and R^{sc} mutants from R^{st} , on the other hand, is more difficult to interpret. X irradiation of the R^{st} allele in pollen decreased paramutagenicity in two instances and effected no change in five others. Evidence from the above mentioned R^{sc}_{132} tests with seed, on the other hand, leaves open the question whether X irradiation may result occasionally in changes in paramutagenicity in the opposite direction also. Most of the observed changes induced by high energy irradiation can be interpreted satisfactorily in terms of loss by deficiency of metameres from a repressor segment adjacent to the R gene. The exceptional results obtained with the R^{sc}_{132} allele, however, are not readily explainable on this simple model of the R system.

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SUMMARY

Evidence showed that when pollen from plants homozygous for either paramutable standard R^r or paramutagenic R^{st} was irradiated with X rays, paramutation was altered in the heterozygotes into which these alleles were subsequently introduced. Paramutation was reduced in 27% of the plants carrying the X-irradiated R^r resulting from the 1,200 r pollen treatment. There was no case, however, in which paramutation was completely lost. 18 and 30% of the treated R^{st} pollen grains from two $R^{st}R^{st}$ plants (out of seven) were significantly reduced in potential paramutagenic action.—Nine R^{sc} alleles were found to be generally insensitive to X irradiation at the seed stage (14,000 r). The allele derived from one of the 68 irradiated seeds was exceptional, however, and showed a possible increase in paramutagenicity. Two paramutagenic $r^r(I)$ alleles were also studied. Paramutation of R^g_6 was reduced in $R^g_6/r^r(I)_5$ heterozygotes when the strongly paramutagenic $r^r(I)_5$ was X-irradiated at the seed stage with 14,000 r. Such a reduction did not occur, however, in R^g_6 heterozygotes carrying the irradiated weakly paramutagenic $r^r(I)_3$ allele. Thus sensitivity to X irradiation appears to vary among different paramutagenic alleles.

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