Previous studies (Brink, Brown, Kermicle and Weyers; Genetics 45:1297-1312, 1960) established that the pigmenting capacities of R^r and eight of its R^g mutant derivatives are reduced to a similar extent in heterozygotes with R^{st}. Testcrosses to $r^g r^g$ pistillate parents of $R^r R^{st}$ and $R^g R^{st}$ sib progeny from $R^r R^g \times R^{st} R^{st}$ matings have confirmed that the R^r and R^g alleles are equally sensitive to the paramutagenic stimulus of R^{st} when measured in terms of aleurone pigmenting action. The present test does not provide for an assessment of the relative paramutagenic potencies of paramutant $R^{r'}$ and paramutant $R^{g'}$. The observation that ten R^g mutants of independent origin do not differ in level of paramutagenic action acquired in heterozygotes with R^{st}, however, agrees with the conclusion, implied by the results of tests of $R^r R^{st}$ and $R^g R^{st}$ plants, that the mutational events underlying the origin of the R^g alleles involved in these studies have not altered the chromosomal elements concerned with paramutation.

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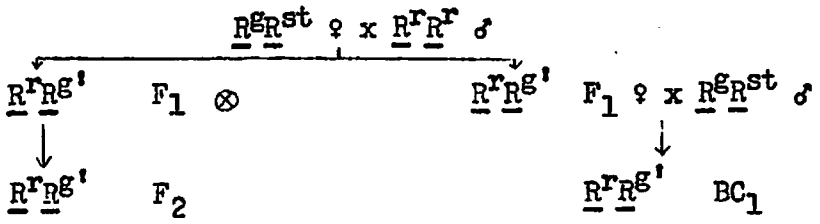
3. Progressive secondary paramutation.

The reduction in the pigmenting action of paramutable R alleles which is induced by paramutant R' genes is slight when compared with that incited by R^{st} (Brown and Brink, Genetics 45:1313-1316). Tests of $R^r R^{g'}$ and $R^{r'} R^g$ plants in F_1, F_2, and backcross 1 generations, which are described here, reveal that the extent of impairment in the pigmenting action of R^g or R^r is cumulative when the paramutant allele acts in two successive sporophytic generations.

$R^r R^{g'}$ staminate testcross parents were produced according to the following mating plan:



Four lines, each containing a different R^g allele, were established from single $R^g R^{st}$ ♀ x $R^r R^r$ ♂ pollinations. A single $R^r R^{g'}$ offspring from each $R^g R^{st}$ x $R^r R^r$ mating was selfed to produce $R^r R^{g'}$ F_2 plants, and another was crossed to $R^g R^{st}$ to produce $R^r R^{g'}$ backcross 1 (BC_1) plants. The two $R^g R^{st}$ plants in each pedigree were sibs grown from the same parental ear.

Table 1

Table 1

Mean scores for $\underline{R}^r\underline{r}^g\underline{r}^g$ kernels from testcrosses of control $\underline{R}^r\underline{R}^g$, $\underline{R}^r\underline{R}^{g'}$ F_1, $\underline{R}^r\underline{R}^{g'}$ F_2, and $\underline{R}^r\underline{R}^{g'}$ BC_1 plants (first four lines of figures), and for $\underline{R}^g\underline{r}^g\underline{r}^g$ kernels from testcrosses of control $\underline{R}^r\underline{R}^g$, $\underline{R}^{r'}\underline{R}^g$ F_1, $\underline{R}^{r'}\underline{R}^g$ F_2, and $\underline{R}^{r'}\underline{R}^g$ BC_1 plants (last four lines of figures). The number of plants tested is given in parentheses following each mean score.

Allele tested	Staminate testcross parent class			
	Control	F_1	F_2	BC_1
R^r	9.05 (6)	8.27 (10)	7.65 (10)	7.36 (10)
R^r	9.26 (6)	8.03 (7)	---	7.29 (7)
R^r	9.05 (6)	8.44 (10)	7.71 (9)	7.65 (10)
R^r	9.31 (6)	8.06 (10)	7.41 (10)	7.16 (10)
R^g_1	9.47 (6)	8.67 (10)	7.84 (10)	7.05 (10)
R^g_3	9.32 (6)	9.38 (10)	8.06 (5)	8.01 (10)
R^g_4	9.20 (6)	8.36 (10)	7.45 (10)	8.57 (10)
R^g_5	9.37 (6)	8.31 (9)	7.87 (4)	7.67 (10)
Mean	9.25	8.44	7.71	7.60
P*		<.001	<.001	<.001

* The probability under the null hypothesis determined by an analysis of variance.

$\underline{R}^{r'}\underline{R}^{g}$ staminate testcross parents were derived by a similar scheme. In this case, four $\underline{R}^{r'}\underline{R}^{g}$ lines differing in the $\underline{R}^{g}$ allele involved were established each from a single $\underline{R}^{g}\underline{r}^{r}$ ♀ x $\underline{R}^{r}\underline{R}^{st}$ ♂ pollination. One $\underline{R}^{r'}\underline{R}^{g}$ plant in each line was selfed and another was pollinated by an $\underline{R}^{r}\underline{R}^{st}$ plant to produce $\underline{R}^{r'}\underline{R}^{g}$ F_2 and $\underline{R}^{r'}\underline{R}^{g}$ BC_1 offspring, respectively.

The progenies of the three matings in each line, along with control $\underline{R}^{r}\underline{R}^{g}$ plants from $\underline{R}^{g}\underline{r}^{r}$ ♀ x $\underline{R}^{r}\underline{R}^{r}$ ♂ crosses, were grown and testcrossed to $\underline{r}^{g}\underline{r}^{g}$ ♀♀ in the same season. Samples of kernels from the testcross ears were first scored for aleurone pigmentation according to an eleven class scale, and then identified as to genotype by seedling test ($\underline{R}^{r}\underline{r}^{g}\underline{r}^{g}$ or $\underline{R}^{r'}\underline{r}^{g}\underline{r}^{g}$ kernels produce red seedlings when germinated; $\underline{R}^{g}\underline{r}^{g}\underline{r}^{g}$ or $\underline{R}^{g'}\underline{r}^{g}\underline{r}^{g}$ kernels produce green seedlings).

The mean scores for $\underline{R}^{r}\underline{r}^{g}\underline{r}^{g}$ kernels from testcrosses of control $\underline{R}^{r}\underline{R}^{g}$, $\underline{R}^{r}\underline{R}^{g'}$ F_1, $\underline{R}^{r}\underline{R}^{g'}$ F_2, and $\underline{R}^{r}\underline{R}^{g'}$ BC_1 plants, and for $\underline{R}^{g}\underline{r}^{g}\underline{r}^{g}$ kernels from testcrosses of control $\underline{R}^{r}\underline{R}^{g}$, $\underline{R}^{r'}\underline{R}^{g}$ F_1, $\underline{R}^{r'}\underline{R}^{g}$ F_2, and $\underline{R}^{r'}\underline{R}^{g}$ BC_1 plants are presented in Table 1. The scores in column 2 of the table represent the non-paramutant level of expression of the various $\underline{R}^{r}$ and $\underline{R}^{g}$ alleles; those in column 3 represent the level of pigmenting action of the same alleles following one generation of heterozygosity with a paramutant factor. It may be seen that the latter values are significantly lower on the average than the former, indicating that, as expected, secondary paramutation has taken place in the F_1 generation $\underline{R}^{r}\underline{R}^{g'}$ and $\underline{R}^{r'}\underline{R}^{g}$ families as a whole.

The mean scores entered in columns 4 and 5 of Table 1, which were obtained from testcrosses of F_2 and BC_1 $\underline{R}^{r}\underline{R}^{g'}$ and $\underline{R}^{r'}\underline{R}^{g}$ plants, indicate the pigmenting capacity of the several $\underline{R}^{r}$ and $\underline{R}^{g}$ alleles after a second generation in combination with a paramutant homologue. Comparisons between the scores in columns 4 and 5 on one hand and those in column 3 on the other, then, provide a test for progressive secondary paramutation in two sporophytic generations. The results are clear. The mean scores obtained by testcrosses of F_2 and BC_1 plants are significantly lower than those obtained by testcrosses of the related F_1 plants. The paramutagenic effects of paramutant $\underline{R}'$ genes, therefore, are persistent and cumulative through at least two successive sporophytic generations.

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