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1. A test for a cytoplasmic component of the R paramutation system.

The stippled allele (R^{st}, spotted aleurone, green seedling) seemingly is unaltered in either aleurone expression or paramutagenic action in R^r R^{st} plants, in which R^r (colored aleurone, red seedling) invariably is changed to a weakly pigmenting, paramutant form, $R^{r'}$. (The tests made, however, would not have disclosed small changes in R^{st} action.)

One possible explanation for this stability of stippled in R^r R^{st} heterozygotes is that R^{st} produces and releases into the cytoplasm a specific product which then mediates the regularly observed change in R^r in the homologous chromosome. The following tests, with W22 inbred stocks, for the occurrence of such a cytoplasmic intermediate substance have given negative results.

Table 1 (See footnotes)

Mating	No. ♂♂ tested on paired ♀♀	Av. color score of R^g $r^r r^r$ kernels only
(1) $r^r r^r$ ♀ x R^g_4 R^g_4 ♂ (control)	14	6.04
(2) $R^{st} r^r$ ♀ x R^g_4 R^g_4 ♂	14	6.19
Difference		0.15
t = 2.206	($t_{.05}$ = 2.160)	Doubtfully significant

[1] r^r = colorless aleurone, red seedling (non-paramutagenic)

[2] R^g_4 = colored aleurone, green seedling, a highly paramutable (in R^g R^{st}) mutant from standard R^r.

[3] R^g_4 $R^{st} R^{st}$ and R^g_4 $r^r r^r$ kernels yield green and red seedlings, respectively, on sprouting, and so are readily separable following scoring for aleurone color.

[4] Kernel color was scored on a scale in which 1 represents colorless and 7 complete pigmentation.

The rationale of the test made is that if $\underline{R}^{st}$ forms a cytoplasmic element of the kind in question then the female gametophytes involved in mating (2), in Table 1, and the derived endosperm, will carry the factor, whereas those concerned in mating (1) will lack it. Accordingly, if the cytoplasmic element promotes paramutation of $\underline{R}^{g}$ in the endosperm, the $\underline{R}^{g}_{-4}\ \underline{r}^{r}\underline{r}^{r}$ kernels from mating (2) will have a lower average color score than those from mating (1). The data in Table 1 show that this is not the result observed. In fact, there is a small, but doubtfully significant difference in the opposite direction, the possible meaning of which will be considered later.

The second experiment, the results of which are summarized in Table 2, was of the same design as the first but the male parents used in pollinating paired ♀♀ in this case were $\underline{R}^{g}_{-6}$' $\underline{R}^{g}_{-6}$' plants. $\underline{R}^{g}_{-6}$' is a comparatively stable paramutant form (extracted initially from $\underline{R}^{g}_{-6}\ \underline{R}^{st}$) of $\underline{R}^{g}_{-6}$, another green seedling mutant from standard $\underline{R}^{r}$. The aleurone pigmenting value of $\underline{R}^{g}_{-6}$', when tested in the conventional way, is about half that of $\underline{R}^{g}_{-6}$ (or $\underline{R}^{g}_{-4}$) on the 1-7 scale. It was thought that $\underline{R}^{g}_{-6}$', by virtue of its known paramutability and inherently much weaker pigmenting action, might be a more sensitive tester for a paramutagenic cytoplasmic element than the $\underline{R}^{g}_{-4}$ allele used in the first experiment.

Table 2

Mating	No. ♂♂ tested on paired ♀♀	Av. color score of $\underline{R}^{g}_{-6}$' $r^{r}r^{r}$ kernels only
(3) $r^{r}\ r^{r}$ ♀ x $\underline{R}^{g}_{-6}$' $\underline{R}^{g}_{-6}$' ♂ (control)	15	2.81
(4) $R^{st}\ r^{r}$ ♀ x $\underline{R}^{g}_{-6}$' $\underline{R}^{g}_{-6}$' ♂	15	3.38
Difference		0.57

$t = 5.072^{**}$ $(t_{(.001)} = 4.140)$ Very highly significant

Again the ($\underline{R}^{g}_{-6}$'$\underline{r}^{r}\underline{r}^{r}$) kernels from the matings in which $\underline{R}^{st}\underline{r}^{r}$ ♀♀ are involved are not less pigmented than kernels of the same genotype from mating (3). On the contrary, they are more darkly colored, on the average, by an amount about equal to one-half class interval on the 1-7 scale. Furthermore, the difference in this case is very highly significant statistically.

Neither experiment (1) nor experiment (2), therefore, affords any evidence that the r^r allele derived from $R^{st}r^r$ plants is accompanied by a cytoplasmic element that depresses the pigment-producing action of a paramutable R gene introduced into the endosperm through the pollen.

The observed small excess in score of $R^g_{-4}\ r^r r^r$ seeds from $R^{st}r^r$ ♀ x $R^g_{-4}\ R^g_{-4}$ ♂ crosses over that from the $r^r r^r$ ♀ x $R^g_{-4}\ R^g_{-4}$ ♂ matings in experiment 1, and the more pronounced difference in the same direction in experiment 2 appear to be meaningful.

A few years ago it was observed that if plants homozygous for one or another R-pale allele (conditioning very dilute aleurone color) were pollinated with $R^{st}\ R^{st}$ the background color on the resulting kernels (i.e., in the areas between the solidly pigmented spots conditioned by R^{st}) often was enhanced, as compared with that of R-pale selfed. The enhancement was not inherited, and so was adjudged not to be due to paramutation of the R-pale gene. The probable explanation of the phenomenon appeared to be that under the action of stippled (considered as an inhibited self-color gene that mutates frequently in the endosperm to the active form) anthocyanin precursors were accumulated in the R^{st}/R-pale/R-pale aleurone cells in amounts above the level characteristic of homozygous R-pale. Additional pigment was then synthesized by R-pale from the added supply of precursors.

The observed intracellular enhancement of aleurone pigment formation by R^{st}, as just described, raised the question whether the phenomenon was expressed in grosser form also as an interaction between stippled kernels and a second class of seeds, on the same ear. This issue was directly relevant to the present experiments because the comparison being made was between r^r-carrying kernels borne on $R^{st}r^r$ and $r^r r^r$ plants, respectively. Evidence has now been obtained that there is, in fact, such a kernel interaction.

Mixtures of pollen were prepared from R^g_{-6}, R^g_{-6}, and $R^{st}\ R^{st}$ plants which were then applied to $r^r r^r$ individuals. The resulting R^g_{-6},$r^r r^r$ kernels were found to be darker, on the average, by a value of 0.27 class intervals on the 1-7 color scale, than R^g_{-6},$r^r r^r$ seeds from $r^r r^r$ ♀ x R^g_{-6}, R^g_{-6}, control matings. The difference was highly significant statistically. It is possibly meaningful also that, whereas the effect of kernels carrying R^{st} in single dose was to raise the color score of the accompanying class of seeds by 0.27 class intervals, the action of the allele in double dose was to increase the corresponding value to 0.57, or about twice as much (Table 2).

This pigment-enhancing "side-effect" of stippled tends, of course, to vitiate the present test for a cytoplasmic component in the R paramutation system. If a cytoplasmic element that depresses R aleurone pigmentation is present in the r^r segregates from $R^{st}r^r$ ♀♀, its effect is exceeded by the oppositely directed inter-kernel action of stippled.

R. A. Brink

2. Relative paramutagenic capacities of the paramutant forms of R^g mutants derived from the standard R^r allele.

It has been found that the standard R^r allele and its R^g mutant derivatives not only become heritably reduced in pigmenting action when passed through a heterozygote with the stippled (R^{st}) allele but that they also acquire the capacity to promote a similar, though smaller, reduction in pigmenting action when combined with other paramutable genes (Brown and Brink, Genetics 45:1313-1316, 1960). The data reported here indicate that ten R^g alleles independently derived by mutation from standard R^r are indistinguishable from one another with regard to the level of paramutagenic activity acquired in heterozygotes with R^{st}.

Pollen from each of twelve R^rR^r plants was applied to silks of R^gR^{st} plants representing the ten R^g alleles. Progeny from a total of 111 successful pollinations of this type were grown in the following season, and two randomly selected R^rR^g' plants from each family were testcrossed to r^gr^g pistillate parents. In this way, R^g genes derived from nine to twelve R^gR^{st} plants in the case of each R^g allele were combined with standard R^r genes from a common source. Differences in paramutagenic competence among the paramutant forms of the various R^g alleles should be reflected in this test as differences in the level of pigmenting action of R^r genes in the corresponding groups of R^rR^g' plants.

Forty-two $R^rr^gr^g$ kernels from each r^gr^g ♀ x R^rR^g' ♂ test mating were scored against a standard set of kernels defining seven pigmentation classes. The mean $R^rr^gr^g$ scores from testcrosses of two R^rR^g' plants from each of the 111 R^gR^{st} x R^rR^r matings are shown in Table 1.

An analysis of variance performed on the data in Table 1 revealed no differences among the mean scores attributable to the R^g alleles involved in the respective pedigrees ($F = .751$, $P > .1$). The overall mean $R^rr^gr^g$ scores from testcrosses of R^rR^g' plants involving individual R^g alleles are all within the range 5.21 to 5.34. These results show that the ten R^g mutants from standard R^r are indistinguishable from one another with regard to the level of paramutagenic action acquired in heterozygotes with R^{st}.