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1. Occurrence of paramutation during endosperm development.

Following Brink's (PNAS 45:819) demonstration that the change of R^r to the paramutant form ($R^{r'}$) does not directly involve zygotene pairing of R^r and R^{st} (stippled aleurone), attempts have been made to demonstrate directly that the alteration occurs in somatic cells. Ashman (Genetics 45:18) derived near-colorless mutants from stippled or R^rR^{st} plants (designated by r^gI or r^rI) which retained the paramutagenic property of R^{st}. Weyers found that the pigment-producing action of the standard R^r allele was not detectably altered in the $R^r/r^rI/r^rI$ endosperms immediately resulting from r^rI/r^rI ♀ X R^rR^r ♂ matings. The present experiment confirms this observation. It is found, however, that if the R allele tested in a comparable way is already a paramutant (R') then its pigment-producing action is further reduced in $R'/r^rI/r^rI$ and $R'/r^gI/r^gI$ endosperms.

Demonstration of this effect of the near-colorless paramutagenic alleles requires the use of R' paramutants which initially have been only slightly reduced in pigment-producing action. The source of R' paramutants for the experiment was, therefore, $R^{scl}R^g$ heterozygotes. The R^{scl} alleles involved were self colored mutants from light stippled which had previously been characterized as being weakly paramutagenic. The R^g allele is a green plant color mutant derived from the standard R^r allele.

The following crosses were made:

W22 ♀♀ parents		W22 ♂♂ parents
r^rI_3/r^g r^r/r^gI_4 r^r/r^g	X	$R^{scl}134/R^g5$ $R^{scl}99/R^g5$ R^g5/R^g5

The r^r and r^g colorless alleles involved in the female parents are nonparamutagenic, while r^rI_3 and r^gI_4 are near-colorless, paramutagenic alleles, with red and green seedling color, respectively.

Single pollen collections were taken from male plants and used to pollinate one ear in each of the three pistillate parents. The resulting kernels were evaluated for level of aleurone pigmentation by separation of the $R^g/-/-$ kernels into classes ranging from 1 (colorless) to 6 (darkly mottled) and 7 (self colored). Kernels were scored individually

for aleurone pigmentation, germinated in order, and the genotype of the endosperm was determined from the seedling color. For examples, in matings involving $\underline{r}^{r}I_3/\underline{r}^{g}$ ♀, the kernels giving red seedlings = $\underline{R}^{g\prime}/\underline{r}^{r}I/\underline{r}^{r}I$ and those giving green seedlings = $\underline{R}^{g\prime}/\underline{r}^{g}/\underline{r}^{g}$. Heterofertilization does not interfere with this classification, since all male parents are homozygous for the green seedling character ($\underline{R}^{sc1}$ alleles are $\underline{R}^{g}$, in Emerson's terminology).

The mean aleurone color scores for the "red" and "green" classes on each ear are based on 50 kernels scored. Table 1 contains these individual class mean values averaged over the number of plants tested, and also the mean difference between "red" and "green" classes per kernel scored.

The full results, of which Table 1 is a summary, are regular. For each of the staminate parents, the difference between classes on individual ears of matings involving $\underline{r}^{r}I/\underline{r}^{g}$ ♀ and $\underline{r}^{r}/\underline{r}^{g}I$ ♀ are in the same direction. The combination involving $\underline{R}^{g\prime}$ with the paramutagenic allele contributed by the female parent is the least pigmented. In the case of the $\underline{R}^{g}5/\underline{R}^{g}5$ staminate parent this difference is extremely small, and is significant only in the case of $\underline{r}^{r}I/\underline{r}^{g}$ ♀ X $\underline{R}^{g}5/\underline{R}^{g}5$ crosses. Even here the observed difference represents a relative displacement of only 18 kernels of the 250 kernels scored for each endosperm genotype, and therefore does not clearly represent a paramutagenic effect of the $\underline{r}^{r}I$ allele.

In contrast the difference between classes is non-significant in all matings involving the $\underline{r}^{r}/\underline{r}^{g}$ pistillate parent.

The reduction in pigmentation level observed for $\underline{R}^{g\prime}/\underline{r}^{r}I/\underline{r}^{r}I$ and $\underline{R}^{g\prime}/\underline{r}^{g}I/\underline{r}^{g}I$ endosperm kernels relative to their respective controls is clearly a consequence of the paramutagenic action of the near-colorless alleles ($\underline{r}^{r}I$ and $\underline{r}^{g}I$). Since it is known that $\underline{R}^{r}$ paramutants from heterozygotes with weakly paramutagenic $\underline{R}^{sc}$ alleles may be further reduced in pigment-producing action if made heterozygous with stippled, the effect observed here may rightly be termed paramutation. It follows, therefore, that the paramutagenic effect of these near-colorless alleles can be manifested in the immediate endosperm phenotype. By extrapolation it appears likely that this secondary alteration of the $\underline{R}^{g\prime}$ paramutants is progressive, and begins as soon as the paramutable $\underline{R}^{g\prime}$ and paramutagenic near-colorless alleles become associated in a common nucleus following fertilization.

The immediate paramutagenic effect of the near-colorless alleles is not detected if the $\underline{R}^{r}$ or $\underline{R}^{g}$ alleles are non-paramutants. There is, therefore, a "threshold" for the effect.

Some $\underline{R}^{sc}$ alleles (mutants from stippled) are non-paramutagenic when standard $\underline{R}^{r}/\underline{R}^{sc}$ heterozygotes are tested in the usual way. Heterozygotes of 3 of these $\underline{R}^{sc}$ alleles with $\underline{R}^{g}5$ were also tested in this

Table 1. Occurrence of paramutation during endosperm development. Mean aleurone color scores for R^{g}'/-/- kernels.

Staminate parent	Number of ♂ plants tested	Pistillate parent(1)	Mean aleurone color scores/kernel(2) "red"(3)	"green"(3)	Mean difference between classes per kernel (red - green)	Significance(4)
$R^{scl_{134}}$/ R^{g5}	6	$r^{r}I/r^{g}$	4.883	5.226	-0.343	**
	6	$r^{r}/r^{g}I$	5.303	4.856	+0.447	*
M^{c757}	6	r^{r}/r^{g}	5.198	5.170	+0.028	-
$R^{scl_{99}}$/ R^{g5}	4	$r^{r}I/r^{g}$	4.535	4.935	-0.40	*
	4	$r^{r}/r^{g}I$	4.330	3.965	+0.365	**
M^{c751}	4	r^{r}/r^{g}	4.685	4.750	-0.065	-
R^{g5}/R^{g5}	5	$r^{r}I/r^{g}$	5.888	5.960	-0.072	*
	5	$r^{r}/r^{g}I$	5.940	5.884	+0.056	-
W 761	5	r^{r}/r^{g}	5.968	5.94	+0.028	-

(1) r^{r} and r^{g} are colorless, non-paramutagenic alleles, while $r^{r}I$ and $r^{g}I$ denote red plant color, near-colorless aleurone, paramutagenic, and green plant color, near-colorless aleurone, paramutagenic, alleles, respectively.

(2) The total number of kernels upon which these mean values are based is given by n X 50 where n is the number of ♂ plants tested.

(3) "red" signifies the class of kernels giving red seedlings on germination and hence of $R^{g}r^{r}r^{r}$ endosperm genotype, and "green" signifies the alternate relation.

(4) * Significant at P = 0.05, ** significant at P = 0.01.

experiment. There is no evidence of an immediate effect of the paramutagenic alleles on $\underline{R}^{g}$ from this source, so that there is no evidence of a subliminal alteration of $\underline{R}^{g}$ in these heterozygotes with non-paramutagenic $\underline{R}^{sc}$ alleles.

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