3. Further evidence about the smoky modifier.

In the 1966 News Letter it was reported that when $\underline{R}^{sk}\underline{r}^{g}$ plants are crossed with $\underline{r}^{g}\underline{r}^{g}$ some of the resulting ears show, besides the expected colorless kernels (genotypically $\underline{r}^{g}\underline{r}^{g}$), two kinds of smoky, darker and lighter, often in equal frequency. Such results could be explained by assuming that the lighter smoky phenotype results from the interaction of $\underline{R}^{sk}$ with a Modifier of the smoky expression that assorts independently of $\underline{R}^{sk}$.

The validity of this assumption can be tested by crossing plants derived from colorless kernels (obtained from the previously mentioned cross) with their dark smoky sibs. In fact, if the smoky Modifier assorts independently of $\underline{R}^{sk}$, approximately one-half of the colorless kernels should carry it. Its presence can be proved by the appearance of two phenotypic classes of smoky, lighter and darker, in the ears obtained from the above mentioned cross. Twenty-four ears so obtained have been scored. Twelve of them segregate only dark smoky and colorless kernels, eight exhibit a clear segregation of dark and light smoky kernels, besides the expected 50% colorless, while the remaining four ears have been discarded because of scoring difficulties. These results clearly indicate that the two classes of smoky are due to the segregation of a smoky Modifier.

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4. Differential response of the R subunits to the paramutagenic action of $\underline{R}^{st}$.

Plant and seed pigments are controlled by the two subunits of the R locus, respectively symbolized P and S. If paramutation is not confined to the S component but affects the R locus as a whole, its expression should be observable also in the sporophytic tissues. In a previous test (M.N.L. 1966) we tried to establish this point by comparing the concentration of pigment extracts of $\underline{r}^{g}\ \underline{R}^{r}$ and $\underline{r}^{g}\ \underline{R}^{r'}$ roots grown on filter paper. The spectrometric determination of root pigments failed to disclose a significant reduction in pigmenting level of paramutant $\underline{r}^{g}\ \underline{R}'$ roots. These data seemed to suggest that the R component conditioning pigment formation in the roots is not significantly affected by its association with a paramutagenic allele. Alternatively, they could simply indicate that no suitable growing conditions for pigment formation were used in our test. In fact, it is likely that the establishment of growth conditions leading to an increased anthocyanin biosynthesis in the sporophytic tissues may make observable even small differences in pigment concentration between $\underline{r}^{g}\ \underline{R}^{r}$ and $\underline{r}^{g}\ \underline{R}^{r'}$ roots.

Such conditions are obtained by allowing seeds to germinate on a medium containing agar and sucrose (0.25%). Furthermore it is possible, using this medium, to extend the measurements of pigment concentration to other tissues like the coleoptile and mesocotyl. This medium has been used to obtain the data that are here presented.

Table 1
Comparison of mean anthocyanin content between:
I. $\underline{r}^{g}\underline{R}^{r}$ and $\underline{r}^{g}\underline{R}^{r'}$ roots II. $\underline{r}^{g}\underline{R}^{r}$ and $\underline{r}^{g}\underline{R}^{r'}$ internodes (1)

Pedigree	Pistillate parent	Genotype	No. individuals tested	$\bar{X}_{(2)}$	s.e.
		I. Internodes			
g607 X gl018	W23	$\underline{r}^{g}\underline{R}^{r}$	85	1.55	0.28
g607 X gl018	W23	$\underline{r}^{g}\underline{R}^{r'}$	85	1.29	0.14
gl013 X gl027	W22	$\underline{r}^{g}\underline{R}^{r}$	60	1.64	0.46
gl013 X gl026	W22	$\underline{r}^{g}\underline{R}^{r'}$	60	1.19	0.35
		II. Roots			
g607 X gl018	W23	$\underline{r}^{g}\underline{R}^{r}$	50	1.08	0.13
g607 X gl018	W23	$\underline{r}^{g}\underline{R}^{r'}$	50	1.13	0.14
gl013 X gl027	W22	$\underline{r}^{g}\underline{R}^{r}$	80	0.60	0.04
gl013 X gl026	W22	$\underline{r}^{g}\underline{R}^{r'}$	80	0.58	0.07

(1) first leaf sheath (2) expressed as mean O.D. at 530 m u

The following crosses were performed to produce the seeds used in this experiment: first, sibs $\underline{R}^{st}\ \underline{r}^{r}$ plants were crossed with a homozygous $\underline{R}^{r}\ \underline{R}^{r}$ stock. Plants of the two genotypic classes so obtained were then used as staminate parents in a cross with $\underline{r}^{g}\ \underline{r}^{g}$ plants. The resulting $\underline{r}^{g}\ \underline{R}^{r}$ and $\underline{r}^{g}\ \underline{R}^{r'}$ kernels were germinated and the amount of pigment in their sporophytic tissues was determined spectrometrically. The results obtained are reported in Table 1. They show that the concentration of pigment extracts from either primary roots or internode tissues of the two classes of seedlings does not differ significantly. The same observations have been extended to $\underline{r}^{g}\ \underline{R}^{r''}$ seedlings. In this second experiment two different $\underline{R}^{st}$ alleles have been used. They represent two sublines derived from two seeds isolated from a homozygous $\underline{R}^{st}/\underline{R}^{st}$ stock. The two alleles differ in their capacity to induce paramutation. The former ($\underline{R}^{st}$-1) is a strong inducer, while the latter ($\underline{R}^{st}$-2) is almost completely devoid of paramutagenic capacity. The comparison in pigment concentration between seedlings of the two classes (see Table 2) shows a significant decrease in only the internode tissues of $\underline{r}^{g}\ \underline{R}^{r''}$ ($\underline{R}^{r}$ ex $\underline{R}^{st-1}$) seedlings.

The last table (Table 3) refers to a test which was performed to establish whether R gene action in the aleurone and in sporophytic tissues is correlated. This test has been performed by choosing, in a sample of $\underline{r}^{g}\ \underline{R}^{r'}$ kernels of common origin, those with the lighter and darker phenotype. Kernels of the two classes, 0-2 nearly colorless and 5-7 nearly colored respectively, have been germinated on the usual medium with addition of Kinetin, and the amount of pigment in their root extracts has

Table 2

Comparison of the mean anthocyanin content between $\underline{r}^{g}\underline{R}r''$ roots and internodes(1) obtained from $\underline{r}^{g}\underline{r}^{g}$ x $\underline{R}^{r'}\underline{R}^{st}$ 1 and $\underline{r}^{g}\underline{r}^{g}$ x $\underline{R}^{r'}\underline{R}^{st}$ 2 matings.

Pedigree	Genotype	Aleurone color classes	No. individuals tested	$\bar{X}$(2)	s.e.	t value
		I. Internodes				
gl222 X gl219-1	$\underline{r}^{g}/\underline{R}''$	0.2	60	0.79	0.06	2.29*
gl222 X gl218-1	$\underline{r}^{g}/\underline{R}''$	5-7	60	0.99	0.06	
		II. Roots				
gl222 X gl219-1	$\underline{r}^{g}/\underline{R}''$	0.2	60	0.79	0.04	0.89 n.s.
gl222 X gl218-1	$\underline{r}^{g}/\underline{R}''$	5-7	60	0.75	0.02	

(1) first leaf sheath and coleoptile

(2) expressed as mean O.D. at 530 m u

*significant at the 5 per cent level.

Table 3

Determination of the mean anthocyanin content of $\underline{r}^{g}\underline{R}^{r'}$ roots obtained from seeds exhibiting different levels of aleurone pigmentation

Genotype	No. individuals tested	Aleurone color classes	Roots mean score (1)	s.e.	t value
$\underline{r}^{g}\ \underline{R}^{r'}$	70	0-2	1.41	0.05	0.77 n.s.
$\underline{r}^{g}\ \underline{R}^{r'}$	70	5-7	1.46	0.05	

(1) expressed as mean O.D. at 530 m u.

been determined. The results of this test indicate that the two kinds of roots do not differ significantly in their pigment content.

The data so far obtained can be summarized as follows:

1. A significant decrease of R action in the plant tissues is obtained only after exposure of paramutable R to the repressive activity of a paramutagenic allele for two successive generations. These results suggest that paramutation, in the plant tissues, is weak and progressive in nature.

2. No corresponding decrease of pigmenting potential is observed in the roots even after two generations of R^r R^{st} heterozygosity.

3. The level of R gene action in the aleurone of paramutable $r^g R'$ individuals is not correlated to its level of action in the roots.

These data suggest that the R locus does not react as a whole to the action of an inducing allele. Rather, it seems that different R subunits react in different ways to the repressive activity of R^{st}. However, the interpretation of these results requires a deeper knowledge of the structural organization of the R region and of the biosynthesis of anthocynanins.

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5. Chromatographic analysis of pigments of the various plant tissues.

A chromatographic analysis of the various pigments has been undertaken with the aim of investigating the following points:

1. Distribution of different pigments in the sporophytic and aleurone tissues of a W22 A_1 A_2 C_1 C_2 Pr R^r b pl stock, hereafter referred to as A C R^r.

2. Chromatographic analysis of the anthocyanins extracted from different tissues of A C R^r plants in order to establish whether they are the same or undergo changes in their chemical composition.

3. Variation in pigment distribution of A C R^r plants carrying various allelic combinations at the R locus.

Table 1 shows the variation of spots in different parts of the tissues of plants genotypically A C R^r. The chromatogram was run first downwards with Butanol--Acetic acid--water (4:1:5) and then from left to right with Acetone--Hydrochloric acid (1:3). Spots 1, 2, 3, 4 are anthocyanins. Spots 5 and 7, faint yellow at the visible light, turn blue after spraying with $FeCl_3$ solution. Spots 8 and 9 react positively with p-toluene sulfonic acid (dark yellow). Spot 10 turns light blue after spraying it with Na_2CO_3 solutions. Spots 15 and 16 react positively with both $AlCl_3$ and Na_2CO_3.